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(54) Title: ENZYME INHIBITORS		
(57) Abstract Isothiourea derivatives and their use in medicine, particularly in the treatment of conditions where there is an advantage in inhibiting nitric oxide synthase, pharmaceutical formulations comprising the same and processes for the preparation thereof are disclosed.		

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ENZYME INHIBITORS

The present invention relates to isothioureia derivatives, to methods for their manufacture, to pharmaceutical compositions containing them and to their use in therapy, in particular their use as nitric oxide synthase inhibitors.

It has been known since the early 1980's that the vascular relaxation brought about by acetylcholine is dependent on the presence of the endothelium and this activity was ascribed to a labile humoral factor termed endothelium-derived relaxing factor (EDRF). The activity of nitric oxide (NO) as a vasodilator has been known for well over 100 years and NO is the active component of amyl nitrite, glyceryl trinitrite and other nitrovasodilators. The recent identification of EDRF as NO has coincided with the discovery of a biochemical pathway by which NO is synthesised from the amino acid L-arginine by the enzyme NO synthase.

NO is the endogenous stimulator of the soluble guanylate cyclase and is involved in a number of biological actions in addition to endothelium-dependent relaxation including cytotoxicity of phagocytic cells and cell-to-cell communication in the central nervous system (see Moncada *et al*, *Biochemical Pharmacology*, **38**, 1709-1715 (1989) and Moncada *et al*, *Pharmacological Review*, **43**, 109-142 (1991)). It is now thought that excess NO production may be involved in a number of conditions, particularly conditions which involve systemic hypotension such as septic (toxic) shock and therapy with certain cytokines.

The synthesis of NO from L-arginine can be inhibited by the L-arginine analogue L-N-monomethyl-arginine (L-NMMA) and the therapeutic use of L-NMMA for the treatment of septic shock and other types of systemic hypotension has been proposed (WO 91/04024 and GB-A-2240041). The therapeutic use of certain other NO synthase inhibitors apart from L-NMMA for the same purpose has also been proposed in WO 91/04024 and in EP-A-0446699.

It has recently become apparent that there are at least three types of NO synthase enzymes as follows:

- (i) a constitutive, Ca^{++} /calmodulin dependent enzyme, located in the endothelium, that releases NO in response to receptor or physical stimulation.

- (ii) a constitutive, Ca^{++} /calmodulin dependent enzyme, located in the brain, that releases NO in response to receptor or physical stimulation.
- (iii) a Ca^{++} independent enzyme which is induced after activation of vascular smooth muscle, macrophages, endothelial cells, and a number of other cells by endotoxin and cytokines. Once expressed this inducible NO synthase synthesises NO for long periods.

The NO released by the constitutive enzymes acts as a transduction mechanism underlying several physiological responses. The function of the NO produced by the inducible enzyme is as a cytotoxic molecule for fighting tumour cells and invading microorganisms (Wright *et al.*, *Card. Res.* 26, 48-57 (1992) and Moncada *et al.*, *Pharmacological Review*, 43, 109-142 (1991)). It also appears that the adverse effects of excess NO production, in particular pathological vasodilation and tissue damage, may result largely from the effects of NO synthesised by the inducible NO synthase.

The NO synthase inhibitors proposed for therapeutic use so far, and in particular L-NMMA, are non-selective in that they inhibit both the constitutive and the inducible NO synthase enzymes. Use of such a non-selective NO synthase inhibitor requires that great care be taken in order to avoid the potentially serious consequences of over-inhibition of the constitutive NO-synthase enzyme including hypertension, thrombosis, CNS toxicity and tissue damage. In particular, in the case of the therapeutic use of L-NMMA for the treatment of septic shock it has been recommended that the patient must be subject to continuous blood pressure monitoring throughout the treatment. Thus, whilst non-selective NO synthase inhibitors have therapeutic utility provided that appropriate precautions are taken, NO synthase inhibitors which are selective in the sense that they inhibit the inducible NO synthase enzyme to a considerably greater extent than the constitutive NO synthase enzyme would be of even greater therapeutic benefit and much easier to use.

The preparation and biological properties of isothioureas have been reported in the literature (Schroeder, *Chem. Revs.*, 1955, 55, 181; Doherty *et al.*, *J. Am. Chem. Soc.*, 1957, 79, 5667; and Brand and Brand, *Org. Synth.*, 1942, 22, 59; Smirk *et al.*, *Brit. Med. J.* 1941, 510-11; *J. Physiol.*, 1942, 100, 474-483; *Lancet*, 1942, 301-303; *J. Physiol.*, 1943, 101, 379-388; Fastier, *Brit. J. Pharmacol.*, 1948, 3, 198). We have now found that isothioureas are inhibitors of NO synthase, and are useful in the treatment of systemic hypotension, and, in particular, the treatment of septic shock. In addition, many of these compounds, possess

selectivity for the inducible NO synthase enzyme as compared with the constitutive NO synthase enzymes.

Accordingly, the present invention provides a method of treatment of conditions requiring inhibition of the nitric oxide synthase enzyme, which comprises administering to a mammal in need thereof an effective amount of an isothiourea derivative having an inhibitory effect against the NO synthase enzyme, or a pharmaceutically acceptable salt thereof. In another aspect, the present invention provides the use of an isothiourea having an inhibitory effect against the NO synthase enzyme for the manufacture of a medicament for the treatment of conditions where there is an advantage in inhibiting the NO synthase enzyme.

More specifically, there is provided a method of treatment of systemic hypotension and/or septic shock which comprises administering to a mammal in need thereof an effective amount of an isothiourea derivative having an inhibitory effect against the NO synthase enzyme, or a pharmaceutically acceptable salt thereof. In a further aspect, there is provided the use of an isothiourea derivative having an inhibitory effect against the NO synthase enzyme for the manufacture of a medicament for the treatment of systemic hypotension and/or septic shock.

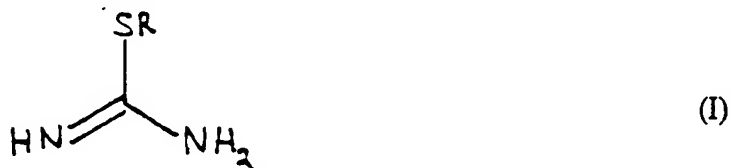
Further conditions where there is an advantage in inhibiting NO production from L-arginine include therapy with cytokines such as TNF, IL-1 and IL-2 or therapy with cytokine-inducing agents, for example 5, 6-dimethylxanthenone acetic acid, and as an adjuvant to short term immunosuppression in transplant therapy. In addition compounds which inhibit NO synthesis may be of use in reducing the NO concentration in patients suffering from inflammatory conditions in which an excess of NO contributes to the pathophysiology of the condition, for example adult respiratory distress syndrome (ARDS) and myocarditis.

There is also evidence that an NO synthase enzyme may be involved in the degeneration of cartilage which takes place in autoimmune and/or inflammatory conditions such as arthritis, rheumatoid arthritis, chronic bowel disease and systemic lupus erythematosus (SLE). It is also thought that an NO synthase enzyme may be involved in insulin-dependent diabetes mellitus. Therefore, a yet further aspect of the present invention provides an isothiourea derivative or salt thereof in the manufacture of a medicament for use in cytokine or cytokine-inducing therapy, as an adjuvant to short term immunosuppression in transplant therapy, for the treatment of patients suffering from inflammatory conditions in which an excess of NO contributes to the pathophysiology of the condition, in autoimmune and/or inflammatory indications and in insulin-dependent diabetes mellitus.

A still further aspect provides a method of treatment of adverse effects associated with cytokine therapy, of short term immunosuppression in transplant therapy, of patients suffering from inflammatory conditions in which an excess of NO contributes to the pathophysiology of the condition, of autoimmune and/or inflammatory indications and of insulin-dependent diabetes mellitus, which comprises administering to a mammal in need thereof an effective amount of an isothioureia derivative having an inhibitory effect against the NO synthase enzyme or a pharmaceutically acceptable salt thereof.

As used herein, reference to "treatment" of a patient is intended to include prophylaxis; the term "mammal" is intended to include a human or an animal.

Preferred isothioureas include those of formula (I)



or a salt thereof, wherein

- R is
- (1) a C₁₋₁₄ hydrocarbyl group; or
 - (2) a 5- or 6-membered heterocyclic ring; or
 - (3) a 9-membered bicyclic heterocyclic ring system


each group R being optionally substituted by one or two groups independently selected from:

- (a) halo;
- (b) -XR¹ wherein

X is oxygen, C(O)_m wherein m is 1 or 2, S(O)_n wherein n is 0, 1, or 2, or NR² wherein R² is hydrogen, C₁₋₆ alkyl or C₃₋₆ cycloalkyl or R² is linked to R¹ to form a C₂₋₆ alkylene group;

R¹ is hydrogen; or C₁₋₆ alkyl, C₂₋₆ alkenyl, C₃₋₆ cycloalkyl, C₇₋₉ aralkyl, C₆₋₁₀ aryl, or a 5- or 6-membered heterocyclic group, each group optionally substituted by one or two groups independently selected

from C₁₋₃ alkyl, hydroxy, C₁₋₃ alkoxy, amino, C₁₋₃ alkylamino, halo, nitro, or a group C(O)_{m'} R^{2b} wherein m' is 1 or 2 and R^{2b} is hydrogen or C₁₋₄ alkyl; or R¹ is a group NR³R⁴ wherein R³ and R⁴ are the same or different and each is hydrogen or C₁₋₄ alkyl or R³ and R⁴ are linked to form a C₂₋₆ alkylene group;


(c) a group (Y)_w-Q-S- wherein

Y is oxygen, S(O)_n wherein n is as hereinbefore defined, or NR⁵ wherein R⁵ is hydrogen or C₁₋₄ alkyl;

w is 0 or 1;

Q is C₂₋₄ hydrocarbyl

or the imino nitrogen is linked to the group R or to the group Q to form a 5- or 6-membered heterocyclic ring;


(d) a group A wherein A is a heterocyclic ring system optionally substituted by a group (Y)_w-Q-S- as hereinbefore defined; or

(e) a C₁₋₆ alkyl, C₂₋₆ alkenyl or alkynyl or C₃₋₆ cycloalkyl group;

or one of the carbon atoms in R is linked to the imino nitrogen atom in the compound of formula (I) to form a 5- or 6- membered heterocyclic ring;

with the proviso that R is not methyl.

Suitably R is

- (1) C₁₋₈ alkyl;
- (2) C₂₋₈ alkenyl or alkynyl;
- (3) a group -(CH₂)_p--(CH₂)_q CH₃ wherein p is 0 to 4 and q is 0 to 3; or
- (4) a 5- or 6- membered heterocyclic ring,

each optionally substituted by one or two groups which may be the same or different selected from

- (a) halo ;

- (b) OR^{2b} wherein R^{2b} is as hereinbefore defined;
- (c) $C(O)_m R^{2b}$ wherein m and R^{2b} are as hereinbefore defined;
- (d) $S(O)_n R^6$ wherein n is as hereinbefore defined and R^6 is C_{1-4} alkyl optionally substituted by one or two groups independently selected from amino or $C(O)_m R^{2b}$ as hereinbefore defined;
- (e) $NR^7 R^8$ wherein R^7 and R^8 are each independently selected from hydrogen, C_{1-4} alkyl, C_{2-4} alkenyl, C_{1-4} alkoxyalkyl; or R^7 and R^8 are linked to form a 5- or 6-membered heterocyclic ring;
- (f) a phenyl ring or a 5- or 6-membered heterocyclic ring each optionally substituted by a group OR^{2b} as hereinbefore defined or by a group $Q-S-\begin{array}{c} \text{NH} \\ \diagup \\ \text{N} \diagdown \\ \text{NH}_2 \end{array}$ wherein Q is as hereinbefore defined; or the imino nitrogen is linked to the group Q to form a thiazole or thiazoline ring; or
- (g) C_{1-4} alkyl when R is a heterocyclic ring;

or one of the carbon atoms in R is linked to the imino nitrogen in the compound of formula (I) to form a thiazole or thiazoline ring.

Most suitably R is

- (1) C_{1-4} alkyl;
- (2) C_{2-4} alkenyl;
- (3) a group $-(CH_2)_p-\text{C}_6\text{H}_4-(CH_2)_q-CH_3$ wherein p is 1 or 2 and q is 0 or 1; or
- (4) a 5- or 6-membered heterocyclic ring containing one or two nitrogen atoms.

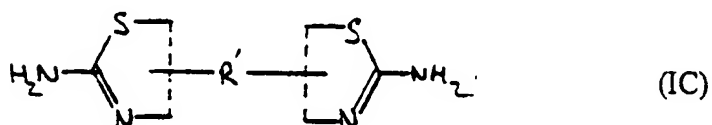
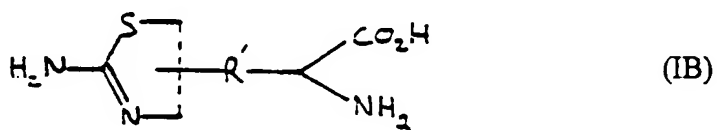
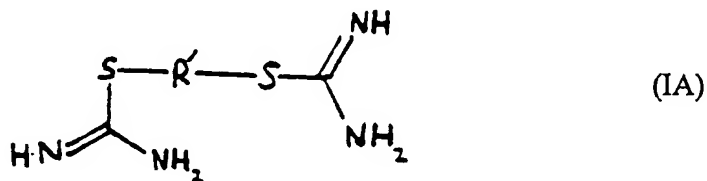
each optionally substituted by one or two groups, which may be the same or different, selected from

- (a) halo, preferably bromo;

- (b) a group $OR^{2b'}$ wherein $R^{2b'}$ is hydrogen or methyl;
- (c) a group $C(O)_m R^{2b'}$ wherein m and $R^{2b'}$ are as hereinbefore defined;
- (d) a group SR^9 wherein R^9 is methyl or ethyl;
- (e) a group $NR^{7b} R^{8b}$ wherein R^{7b} and R^{8b} are independently selected from hydrogen or C_{1-4} alkyl, preferably hydrogen, methyl or ethyl;
- (f) a phenyl ring optionally substituted by a group $OR^{2b'}$ or a group $Q-S-\begin{array}{c} \text{NH} \\ \diagup \\ \text{NH}_2 \end{array}$ as hereinbefore defined;
- (g) a 5- or 6-membered heterocyclic ring containing one or two heteroatoms independently selected from nitrogen or oxygen; or
- (h) C_{1-4} alkyl, preferably methyl

or one of the carbon atoms in R is linked to the imino nitrogen in the compound of formula (I) to form a thiazole or thiazoline ring.

Formula (I) includes isothiourea derivatives of formula (IA), (IB) and (IC)

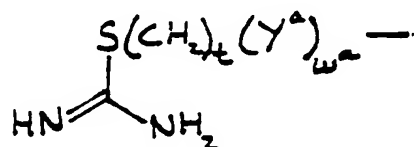


wherein R' is a C_{1-8} alkylene group or a C_{2-8} alkenylene or alkynylene group each optionally containing a phenyl ring, a 5- or 6-membered heterocyclic ring or a group X as hereinbefore defined, and the dotted line represents a double or a single bond.

Formula I also includes compounds of formula (II)



or a salt thereof, wherein R^a is a C_{1-8} hydrocarbyl or 5- or 6-membered heterocyclic ring or a 9-membered bicyclic heterocyclic ring system each optionally substituted by halo or by one or two groups $-\text{X}^a\text{R}^{1a}$ wherein R^{1a} is hydrogen, C_{1-6} alkyl, C_{3-6} cycloalkyl, C_{7-9} aralkyl, C_{6-10} aryl, or a 5- or 6-membered heterocyclic group each optionally substituted by C_{1-3} alkyl, C_{1-3} alkoxy, amino, halo or nitro or R^{1a} is a group $\text{NR}^{3a}\text{R}^{4a}$ wherein R^{3a} and R^{4a} are the same or different and each is hydrogen or C_{1-3} alkyl or R^{3a} and R^{4a} are linked to form a C_{2-6} alkylene group and X^a is oxygen, $\text{C}(\text{O})_m^a$ wherein m^a is 1 or 2, $\text{S}(\text{O})_n^a$ wherein n^a is 0, 1 or 2 or NR^{2a} wherein R^{2a} is hydrogen, C_{1-6} alkyl or C_{3-6} cycloalkyl or R^{2a} is linked to R^{1a} to form a C_{2-6} alkylene group, or by a group



wherein t is 0 to 4 and w^a is 0 or 1, Y^a is oxygen, sulphur and NR^{7a} wherein R^{7a} is hydrogen or C_{1-4} alkyl:

or R^a links the sulphur atom to one of the nitrogen atoms in the compound of the formula (I) to form a 5- or 6-membered heterocyclic ring, with the proviso that R^a is not methyl.

One preferred group of compounds are those wherein R is not methyl, ethyl, propyl or isopropyl.

Preferred compounds of the formula (I) include:

- S-(2-aminoethyl)isothiourea
- S-(2-(dimethylamino)propyl)isothiourea
- S-(2-methyl-2-propenyl)isothiourea
- S,S'-ethylenebis(isothiourea)
- S,S'-pentamethylenebis(isothiourea)
- S-(2-(dimethylamino)ethyl)isothiourea

2-amino-2-thiazoline
S,S'- hexamethylenebis(isothiourea)
S,S'- heptamethylenebis(isothiourea)
S-benzylisothiourea
S-(2-morpholinoethyl)isothiourea
S-(6-methyl-2-(methylthio)-4-pyrimidinyl)isothiourea
S,S'-(1,4-phenylenebis(methylene))diisothiourea
S-tertbutylisothiourea
S-(4-ethylbenzyl)isothiourea
S-((methylthio)methyl)isothiourea
S-(3-bromopropyl)isothiourea
S-(2-bromoethyl)isothiourea
S-(3-methyl-2-butenyl)isothiourea
S-allylisothiourea
S-(3-aminopropyl)isothiourea
S,S'-(1,3-phenylenebis(methylene))diisothiourea
S,S'-(2-methylene-1,3-propanediyl) diisothiourea
S,S'-(2-butyne-1,4-diyl)diisothiourea
S,S'-(1,3-phenylenebis(1,2-ethanediyl))diisothiourea
S,S'-(1,4-phenylenebis(1,2-ethanediyl))diisothiourea
2-amino-5-methylthiazole
S-((2-amino-4-thiazolyl)methyl)-L-cysteine
3((2-amino-4-thiazolyl)methyl)-L-alanine
2-amino-4-methylthiazole
2-amino-4,5-dimethylthiazole
S-(2-(1H-pyrrol-1-yl)ethyl)isothiourea
S-(3-hydroxypropyl)isothiourea
S-(2-(phenyl)ethyl)isothiourea
S-(2-(3-methoxyphenyl)ethyl)isothiourea
4-((2-amino-4-thiazolyl)methyl)-L-homoalanine
N,N-1,3,phenylenebis(methylene))bis(S-methylisothiourea)
N,N-(1,3-phenylenebis(methylene))bis(S-ethylisothiourea)
S-(2-(5-((amidinothio)methyl)-2-thienyl)ethyl)isothiourea
S-(3-(4-((amidinothio)methyl)phenyl)propyl)isothiourea
S-(3-(5-(2-amidinothio)ethyl)-2-thienyl)propyl)isothiourea
S-(2-(4-fluorophenyl)ethyl)isothiourea
S-(2-(4-bromophenyl)ethyl)isothiourea

S-(2-(3-methoxyphenyl)ethyl)isothiourea
S-(2-(3-methylphenyl)ethyl)isothiourea
S-(2-(4-ethoxyphenyl)ethyl)isothiourea
S-(2-(4-methoxyphenyl)ethyl)isothiourea
S-(2-(2-bromophenyl)ethyl)isothiourea
S-(2-(2-fluorophenyl)ethyl)isothiourea
S-(2-(3-nitrophenyl)ethyl)isothiourea
S-(3-(1H-pyrrol-1-yl)propyl)isothiourea
S-(2-(2-chlorophenyl)ethyl)isothiourea
S-(2-(2,5-dimethylphenyl)ethyl)isothiourea
S-(2-(4-ethoxy-3-methoxyphenyl)ethyl)isothiourea

and salts thereof.

Especially preferred compounds include S,S'-(1,3-phenylenebis(1,2-ethanediyl))diisothiourea, S,S'-(1,4-phenylenebis(1,2-ethanediyl)) diisothiourea, S-(2-(5-amidinothio)methyl)-2-thienyl)ethyl)isothiourea, S-(3-(5-(2-amidinothio)ethyl)-2-thienyl)propyl)isothiourea and S-(2'-(3-methoxyphenyl) ethyl)isothiourea.

Other preferred compounds for the treatment of Septic Shock are S-ethylisothiourea, S-propylisothiourea and S-isopropylisothiourea, particularly S-ethylisothiourea and S-isopropylisothiourea, and especially S-ethylisothiourea.

By the term "hydrocarbyl" group is meant a group that contains only carbon and hydrogen atoms but may contain double and/or triple bonds and which may be cyclic or aromatic in nature.

By the term "heterocyclic ring" is meant a cyclic compound containing one to three hetero atoms selected from oxygen, sulphur and nitrogen, and preferably nitrogen or sulphur.

By the term "halo " is meant fluoro, chloro, bromo or iodo, and preferably bromo.

The compounds of formula (I) may include a number of asymmetric centres in the molecule depending on the precise meaning of the various groups and formula (I) is intended to include all possible isomers.

In a further aspect the present invention provides an isothiourea of the formula (I) other than benzylisothiourea, S,S-(1,4-phenylenebis(methylene))diisothiourea and S-(2-(dimethylamino)ethyl)isothiourea, or a pharmaceutically acceptable salt thereof having an inhibitory effect against the NO synthase enzyme for use in medicine.

In another aspect the present invention provides novel compounds of the formula (IA), (IB) and (IC) as hereinbefore defined. Such compounds include:

S,S'-(1,4-phenylenebis(1,2-ethanediyl))diisothiourea
S-(2-(1H-pyrrol-1-yl)ethyl)isothiourea
S-((2-amino-4-thiazolyl)methyl)-L-cysteine
 γ -(2'-amino-4-thiazolyl)-L-homoalanine
S,S'-(1,2-phenylenebis(1,2-ethanediyl))diisothiourea
 β -(2'-amino-4'-thiazolyl)-L-alanine
S-(2'-amino-5'-(R,S)-thiazolinylmethyl)-L-cysteine
4-((2-amino-4-thiazolyl)methyl)-L-homoalanine
N,N-(1,3-phenylenebis(methylene))bis(S-methylisothiourea)
N,N-(1,3-phenylenebis(methylene))bis(S-ethylisothiourea)
S-(3-(4-((amidinothio)methyl)phenyl)propyl)isothiourea
S-(2-(5-((amidinothio)methyl)-2-thienyl)ethyl)isothiourea
S-(3-(5-(2-amidinothio)ethyl)-2-thienyl)propyl)isothiourea
S-((2-amino-4-thiazolyl)methyl)-D-cysteine
S-((2-amino-4-thiazolyl)methyl)-(D,L)-homocysteine
S-(2-(2-amino-4-thiazolyl)ethyl)-L-cysteine
S-(2-(4-fluorophenyl)ethyl)isothiourea
S-(2-(4-bromophenyl)ethyl)isothiourea
S-(2-(3-methoxyphenyl)ethyl)isothiourea
S-(2-(3-methylphenyl)ethyl)isothiourea
S-(2-(4-ethoxyphenyl)ethyl)isothiourea
S-(2-(4-methoxyphenyl)ethyl)isothiourea
S-(2-(2-bromophenyl)ethyl)isothiourea
S-(2-(2-fluorophenyl)ethyl)isothiourea
S-(2-(3-nitrophenyl)ethyl)isothiourea
S-(3-(1H-pyrrol-1-yl)propyl)isothiourea
S-(2-(4-ethoxy-3-methoxyphenyl)ethyl)isothiourea
S-(2-(2,4,6-trimethylphenyl)ethyl)isothiourea

S-(2-(2,6-dimethoxyphenyl)ethyl)isothiourea

and salts thereof.

The present invention includes isothioureas in the form of salts, in particular acid addition salts. Suitable salts include those formed with both organic and inorganic acids. Such acid addition salts will normally be pharmaceutically acceptable although salts of non-pharmaceutically acceptable salts may be of utility in the preparation and purification of the compound in question. Thus, preferred salts include those formed from hydrochloric, hydrobromic, sulphuric, citric, tartaric, phosphoric, lactic, pyruvic, acetic, trifluoroacetic, succinic, oxalic, fumaric, maleic, oxaloacetic, methanesulphonic, ethanesulphonic, p-toluenesulphonic, benzenesulphonic and isethionic acids. Salts of isothioureas can be made by reacting the appropriate compound in the form of the free base with the appropriate acid.

Whilst it may be possible for the isothioureas of the present invention to be administered as the raw chemical, it is preferable to present them as a pharmaceutical formulation. According to a further aspect, the present invention provides a pharmaceutical formulation comprising an isothiourea of the present invention or a pharmaceutically acceptable salt or solvate thereof, together with one or more pharmaceutically acceptable carriers therefor and optionally one or more other therapeutic ingredients, for example an antibiotic, and/or a volume replacement liquid. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The formulations include those suitable for oral, parenteral (including subcutaneous, intradermal, intramuscular, intravenous and intraarticular), rectal and topical (including dermal, buccal, sublingual and intraocular) administration although the most suitable route may depend upon for example the condition and disorder of the recipient. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into association a compound of formula (I) or a pharmaceutically acceptable salt or solvate thereof ("active ingredient") with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both and then, if necessary shaping the product into the desired formulation.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, lubricating, surface active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein.

Formulations for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example, saline, water-for-injection, immediately prior to use. Alternatively, the formulations may be presented for continuous infusion.

Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Formulations for rectal administration may be presented as a suppository with the usual carriers such as cocoa butter or polyethylene glycol.

Formulations for topical administration in the mouth, for example buccally or sublingually, include lozenges comprising the active ingredient in a flavoured basis such as sucrose and acacia or tragacanth, and pastilles comprising the active ingredient in a basis such as gelatin and glycerin or sucrose and acacia.

Preferred unit dosage formulations are those containing an effective dose, as herein below recited, or an appropriate fraction thereof, of the active ingredient.

It should be understood that in addition to the ingredients particularly mentioned above, the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavouring agents.

For each of the aforementioned conditions, the compounds of the invention may be administered orally or via injection at a dose of from 0.1 to 250mg/kg per day. The dose range for adult humans is generally from 5mg to 17.5g/day, preferably 5mg to 2g/day and most preferably 10mg to 1g/day. Tablets or other forms of presentation provided in discrete units may conveniently contain an amount of compound of the invention which is effective at such dosage or as a multiple of the same, for instance, units containing 5mg to 500mg, usually around 10mg to 200mg.

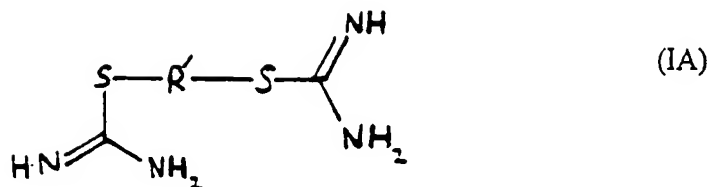
The compounds of formula (I) are preferably administered orally or by injection (intravenous or subcutaneous). The precise amount of compound administered to a patient will be the responsibility of the attendant physician. However the dose employed will depend on a number of factors, including the age and sex of the patient, the precise disorder being treated, and its severity. Also the route of administration may vary depending on the condition and its severity.

The present invention also provides processes for the preparation of novel compounds as hereinbefore defined, analogous to those known in the art for preparing isothiurea derivatives.

Thus, compounds of formula (I) or protected derivatives thereof may be prepared by the reaction of thiourea with a compound $RL(L')_r$ wherein R is as hereinbefore defined, L and L' are both leaving groups, for example a halo atom such as bromo, and r is 0 or 1, followed by deprotection if necessary.

More specifically,

- (i) compounds of formula (IA)

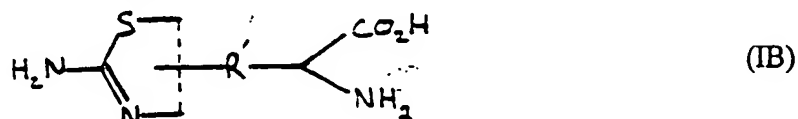


as hereinbefore defined, may be prepared by the reaction of thiourea with a compound LR'L' wherein L,L' and R' are as hereinbefore defined. Suitably the reaction is carried out in a polar solvent, such as ethanol, at a temperature of from 20°C to the refluxing solvent temperature.

Compounds of the formula LR'L' are commercially available or may be prepared from the corresponding diol, HO-R'-OH, suitably by reaction in a polar solvent such as dichloromethane in the presence of a halogenating agent such as carbon tetrabromide and triphenylphosphine.

Compounds of formula HO-R'-OH are commercially available or may be prepared by methods known in the art.

(ii) Compounds of formula (IB)

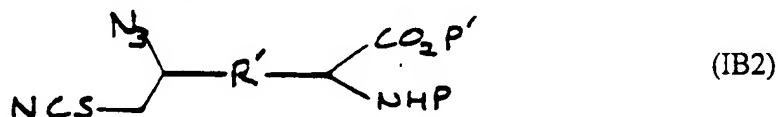


as hereinbefore defined may be prepared by deprotection of a compound of formula (IB1)



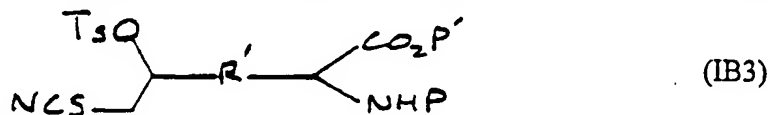
wherein R' and the dotted line are as hereinbefore defined, and P and P' are the same or different and are both protecting groups such as benzyl, benzyloxycarbonyl or tert-butoxycarbonyl. The reaction may be carried out in trifluoroacetic acid at a non-extreme temperature of from -20°C to 100°C such as 0°C in the presence of scavenger molecules such as thioanisole and 1,2-ethanedithiol.

When the dotted line represents a single bond and the substituent is in the 4-position, compounds of formula (IB1) may be prepared from a compound of formula (IB2)



wherein R', P and P' are as hereinbefore defined. The reduction of azide to amine and cyclization to thiazoline may be carried out in tetrahydrofuran in the presence of triphenylphosphine.

Compounds of formula (IB2) may be prepared from compounds of formula (IB3)



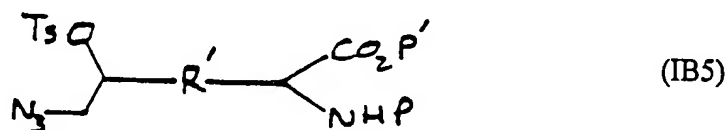
wherein R, P and P' are as hereinbefore defined, by displacement of tosylate with azide anion in a polar solvent such as dimethyl formamide at non-extreme temperature of from -20°C to 200°C such as the refluxing solvent temperature.

Compounds of formula (IB3) may be prepared from compounds of formula (IB4)



wherein R', P' and P are as hereinbefore defined, by the addition of thiocyanate anion to yield a ring-opened alkoxide which may be trapped as the tosyl derivative (IB3) by the addition of para-toluenesulphonyl chloride. Compounds of formula (IB4) may be prepared by methods known to a person skilled in the art.

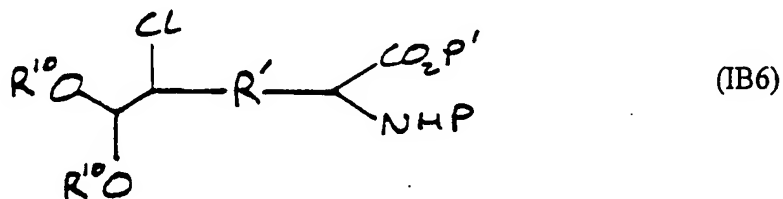
When the dotted line represents a single bond and the substituent is in the 5-position compounds of formula (IB1) may be prepared from a compound of formula (IB5)



by the displacement of tosylate with thiocyanate anion (Tetrahedron Asymmetry 1992, 749-752), which may be carried out in a polar solvent such as ethanol at non-extreme temperatures, of from -20°C to 200°C such as the refluxing solvent temperature, followed by a cyclisation by methods analagous to those described for the preparation of compounds of formula (IB) from those of formula (IB2). Compounds of formula (IB5) may be prepared from the corresponding epoxide by ring opening with azide anion followed by trapping of the alkoxide by para-toluenesulphonyl chloride in a polar solvent such as

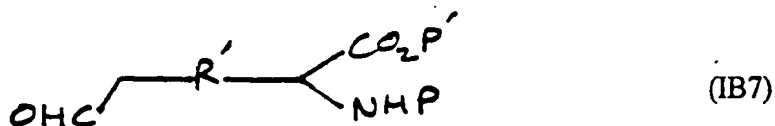
dimethylformamide at non-extreme temperatures of from -20°C to 200°C such as 100°C (Tetrahedron Lett. 1990, 31 (2), 221).

When the dotted line represents a double bond and the substituent on the thiazole ring is in the 5-position, compounds of formula (IB1) may be prepared by the cyclisation of chloroacetals of formula (IB6)



wherein R', P and P' are as hereinbefore defined and R¹⁰ is a C₁₋₄ alkyl group, with thiourea. The reaction may be carried out in a polar solvent such as acetone or ethanol at a non-extreme temperature of from -20°C to 200°C (Chem. Abs. 54:14230d).

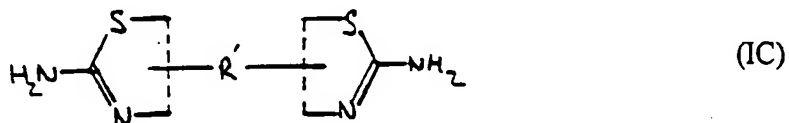
Compounds of formula (IB6) may be prepared from sulphuryl chloride and aldehydes of formula (IB7)



wherein R', P and P' are as hereinbefore defined (Proc. Indian. Acad. Sci. 1941, 14A, 630-5; Chem Abs. 36: 410z). Compounds of formula (IB7) are commercially available or may be prepared by methods known to a person skilled in the art.

When the dotted line represents a double bond and the substituent on the thiazole ring is in the 4-position, compounds of formula (IB1) may be prepared by methods known in the art, for example α-N-benzyloxycarbonyl-β-(2'-amino-4'-thiazolyl) alanine benzyl ester (Synthetic Communications 1990, 20 (30), 3097-3102).

(iii) Compounds of formula (IC)



may be prepared:

- (a) When the dotted line represents a single bond and the substituent is in the 4-position, by methods analogous to those described for the preparation of compounds of formula (IB) from those of formula (IB4).
- (b) When the dotted line represents a single bond and the substituent is in the 5-position of the thiazoline ring, by methods analogous to those described for the preparation of compounds of formula (IB) from those of formula (IB5)
- (c) When the dotted line represents a double bond and the substituent is in the 4-position of the thiazole ring, by the reaction of a compound of formula (IC1)



with thiourea. Suitably the cyclisation reaction may be carried out in a polar solvent such as acetone at a non-extreme temperature of from -20°C to 200°C such as 20°C .

Compounds of formula (IC1) may be prepared from compounds of formula $\text{HO}_2\text{C}-\text{R}'-\text{CO}_2\text{H}$, wherein R' is as hereinbefore defined by methods known in the art (J. Chem. Soc. 1940, 1304-7; Chem. Abs. 35: 113³).

- (d) When the dotted line represents a double bond and the substituent is in the 5-position of the thiazole ring, by methods analogous to those described for the preparation of a compound of formula (IB) from a compound of formula (IB6).

The activity of compounds of the formula (I) as inhibitors of isolated NO synthase enzymes has been demonstrated against NO synthase enzymes isolated from the human placenta, brain and cytokine-induced carcinoma cells.

The present invention will now be described by way of example only:-

Example 1

Preparation of S,S'-(1,4-Phenylenebis(1,2-ethanediy))
diisothiourea dihydrobromide

A solution of 1,4-phenylenediacetic acid (10.0g, 51.5 mmol) in tetrahydrofuran (200mL) was added dropwise to a 0°C stirred suspension of lithium aluminium hydride (3.91g, 103 mmol) in tetrahydrofuran (30mL). The mixture was warmed to reflux for 3 hours and then cooled to 0°C. The excess lithium aluminium hydride was quenched by the slow addition of water (4.0mL), 15% sodium hydroxide (4.0mL), and water (12mL). The suspension was stirred with magnesium sulphate for five minutes, filtered and concentrated. The crude oil was purified by silica gel chromatography (ethyl acetate/hexanes gradient, 50-100%) to provide intermediate diol (7.38g, 86%) as a clear, colourless oil. A solution of this oil in dichloromethane (200mL) at 0°C was treated with carbon tetrabromide (32.4g, 97.7mmol) and triphenylphosphine (25.6g, 97.7 mmol). The mixture was stirred at 20°C for four hours before pentane (500mL) was added. After standing for 15 hours, the solution was decanted from a brown-coloured solid, concentrated and purified by silica gel chromatography (ethyl acetate/hexanes gradient 0-20%) to yield 1,4-phenylenebis(1,2-ethanediyl) dibromide (8.2g, 64%) as an oil. A solution of the dibromide (4.0g, 13.7mmol) and thiourea (2.09g, 27.4mmol) in absolute ethanol (100mL) was refluxed for 2 hours, cooled and concentrated to dryness. The crude solid was recrystallized from ethanol to yield 1,4-phenylenebis(1,2-ethanediyl)diisothiurea dihydrobromide (2.89g, 47%) as a white crystalline solid. M.p. = 234-236°C.

¹H NMR (200 MHz, D₂O) δ 7.26 (s, 4H), 3.38 (t, J=7.0 Hz, 4H), 3.01 (t, J=7.0 Hz, 4H).

Anal. Calcd. for C₁₂H₂₀Br₂N₄S₂: C, 32.44; H, 4.54; Br, 35.97; N, 12.61; S, 14.43.

Found: C, 32.45; H, 4.59; Br, 35.89; N, 12.51; S, 14.35.

Example 2

Preparation of S-((2-amino-4-thiazolyl)methyl)-L-cysteine

To a solution of 2-amino-4-chloromethylthiazole (Sprague, J.M. et al. J. Am. Chem.Soc. 1946, 68, 2155-2159) (1.0g, 5.4mmol) and L-cysteine hydrochloride (804 mg, 5.1 mmol) in dimethylformamide (10ml) was added potassium carbonate (2.5 g). The suspension was stirred at 22°C for 18 hours. The resulting mixture was concentrated to dryness, redissolved into water, and loaded onto an ion-exchange column (Dowex 50X8, strongly acidic). The product was eluted with a gradient of ammonium hydroxide (0.1N to 0.5N). The pooled product fractions were partially concentrated and freeze-dried to yield S-(2'-amino-4'-thiazolylmethyl)-L-cysteine (1.04g, 87%) as a tan-coloured, electrostatic solid.

¹H NMR (300 MHz, D₂O) δ 6.5 (s, 1H), 3.8 (m, 1H), 3.6 (s, 2H), 3.05-2.85 (m, 2H).

Mass Spectrum (CI) 234 (M + 1, 71%).

Example 3

Preparation of γ -(2'-amino-4'-thiazolyl)-L-homoalanine
(3-((2-Amino-4-thiazolyl)methyl)-L-alanine)

γ -(2'-amino-4'-thiazolyl)-L homoalanine was prepared from α -N-t-butoxycarbonyl- γ -(2'-amino-4'-thiazolyl)-L-homoalanine benzyl ester (Patt et al. Synth.Comm. 1990, 20 (20), 3097-3102) in 9.6% yield, according to the method of Example 6.

^1H NMR (D_2O) δ 6.5 (s, 1H), 3.9 (t, J=6.4 Hz), 2.75 (m, 2H), 2.2 (m, 2H).

Anal. Calcd. for $\text{C}_7\text{H}_{11}\text{N}_3\text{O}_2\text{S}\cdot\text{C}_2\text{HF}_3\text{O}_2\cdot 0.3\text{H}_2\text{O}$: C, 29.31; H, 2.89; N, 8.54; S, 6.52.
Found: C, 29.37; H, 3.02; N, 8.53; S, 6.63.

Example 4

Preparation of S,S'-(1,3-phenylenebis(1,2-ethanediyl))
diisothiourea dihydrobromide

S,S'-(1,3-phenylenebis(1,2-ethanediyl))diisothiourea dihydrobromide was prepared according to the method of Example 1 from 1,3-phenylenediacetic acid (Aldrich). Recrystallization from ethanol gave a white crystalline solid (m.p. 194-190°C).

^1H NMR (200 MHz, D_2O) δ 7.4-7.2 (m, 4H), 3.39 (t, J=6.9 Hz, 4H), 3.02 (t, J=6.9 Hz, 4H).

Anal. Calcd. for $\text{C}_{12}\text{H}_{20}\text{Br}_2\text{N}_4\text{S}_2$: C, 32.44; H, 4.54; Br, 35.97; N, 12.61; S, 14.43.

Found: C, 32.52; H, 4.49; Br, 36.04; N, 12.61; S, 14.35.

Example 5

Preparation of S,S'-(1,2-Phenylenebis (1,2-ethanediyl)) diisothiourea dihydrobromide

S,S'-(1,2-phenylenebis(1,2-ethanediyl))diisothiourea dihydrobromide was prepared from 1,2-phenylenediacetic acid (Aldrich) in 30% overall yield as a yellow foam according to the method of Example 1.

^1H NMR (300 MHz, D_2O) δ 7.29 (s, 4H), 3.38 (t, J=7.0 Hz, 4H), 3.09 (t, J=7.0 Hz).

Recrystallization from ethanol gave an analytical sample, m.p. = 205-207°C.

Anal. Calcd. for $\text{C}_{12}\text{H}_{20}\text{Br}_2\text{N}_4\text{S}_2$: C, 32.44; H, 4.54; Br, 35.97; N, 12.61; S, 14.43.

Found: C, 32.47; H, 4.58; Br, 35.92; N, 12.58; S, 14.43.

Example 6

Preparation of β -(2'-amino-4'-thiazolyl) alanine
(3-(2-amino-4-thiazolyl)-L-alanine)

To a stirred solution of α -N-t-butoxycarbonyl- β -(2'-amino-4'-thiazolyl)alanine benzyl ester (1.47g) (Patt et al., Synth. Commun. 1990, 20 (20), 2097-3102) in dichloromethane (15ml) at -88°C under a nitrogen atmosphere was added triethyl silane (3ml) followed by trifluoroacetic acid (3ml). The solution was warmed to room temperature over one hour before concentrating. The residue was treated with acetic acid (30mL), 20% Pd/C (2.0g), and 1,4-cyclohexadiene (20mL). The mixture was sonicated from room temperature to 33°C over 2 hours and filtered through celite washing with water. The filtrate was concentrated and redissolved into 30 mL of acetic acid and treated with 2.0 grams of fresh 20% Pd/C and 2mL of 1,4-cyclohexadiene. After sonnicating for two hours, the suspension was filtered through celite. This process of adding fresh catalyst was repeated three times before the crude product was purified by repetative semi-prep reverse phase chromatography (C-18, elution with 10% methanol/water contining 0.1 % triluoroacetic acid). Freeze-dried product fractions gave 337 mg (18%) of β -(2'-amino-4'-thiazolyl)-L-alanine as a sticky glass-like solid.

^1H NMR (D_2O) δ 6.6 (s, 1H), 4.0 (t, $J=7.6$ Hz, 1H), 3.15 (m, 2H). Mass spectrum (CI) 188 (M+1).

Anal. Calcd. for $\text{C}_{12}\text{H}_{12}\text{N}_3\text{F}_9\text{O}_8\text{S}$: C, 27.23; H, 2.29; N, 7.94; S, 6.06.

Found: C, 27.22; H, 2.42; N, 7.8; S, 5.67.

Example 7

Preparation of S, S'-(2,6-pyridylenebis(methylene))diisothiourea

To a solution of 2,6- pyridinedimethanol (5.0g, 35.9 mmol) in dichloromethane (200ml) at 0°C was added carbon tetrabromide (23.83g, 71.9 mmol) and triphenylphosphine (18.85 g, 71.9 mmol). The solution was stirred with warming to 20°C over six hours. Pentane (300mL) was added. The solution was allowed to stand at 20°C for 16 hours before filtering to remove solid impurites. The filtrate was concentrated to an oil that was purified by silica gel chromatography (hexanes, then 10% ethyl acetate/hexanes) to give 2,6-pyridinedibromide (4.47, 47%) as an off-white powdery solid. To a solution of 2,6-pyridinedibromide (3.94 g, 14.87mmol) in ethanol (100ml) was added thiourea (2.26g, 29.74 mmol), and the resulting suspension was stirred at reflux for two hours. The solution was concentrated to dryness to yield S,S'(2,6-pyridylenebis(methylene)) diisothiourea (5.3g, 83.9%) as a white powder.

^1H NMR (200 MHz, DMSO) δ 7.95 (t, $J=7.6$ Hz, 1H), 7.51 (d, $J=7.8$ Hz, 2H), 4.65 (s, 4H).

Anal. Calcd for $C_9H_{15}N_5S_2Br_2 \cdot 0.6 H_2O$: C, 25.26; H 3.81; N, 16.36; S, 14.98; Br, 37.34
Found: C, 25.3; H, 3.78; N, 16.25; S, 14.94; Br, 37.38.

Example 8

Preparation of S-(2'-amino-5'-(R,S)-thiazolinylmethyl)-L-cysteine

To a solution of 2-amino-5-iodomethylthiazoline (4.44g, 12mmol) (Creeke & Mellor, Tet. Lett. 1989, 30 (33), 4435-4438) and L-cysteine hydrochloride (1.76g, 10.0mmol) in dimethylformamide (75 ml) was added potassium carbonate (5.0g 36mmol). The suspension was stirred at 22°C for 72 hours and refluxed for 30 minutes. Acetonitrile was added and the mixture filtered. The solids obtained were washed repeatedly with warm methanol. Dilution of the methanol solutions with ethanol produced a white precipitate that was removed by filtration. The concentrated filtrate (oil) was taken up into methanol/ethanol and treated with ethanolic hydrogen chloride until no further precipitation was observed and the mixture was filtered. The oil resulting from concentration of the filtrate was purified by ion-exchange chromatography (Dowex 50X8, strongly acidic) eluting with 0.1N ammonium hydroxide. The ninhydrin positive fractions were pooled and freeze-dried to yield 0.453g of a light tan-coloured solid contaminated with dimethylformamide. This solid was purified by preparative HPLC (C18 reverse phase, methanol:water:trifluoroacetic acid/5:95:0.1) to yield 0.36g of a S-(2'-amino-5'-(R, S)-thiazolinylmethyl)-L-cysteine as the trifluoroacetate salt. TLC (ammonium hydroxide: methanol/ 1:50) $R_f = 0.5$. 1H NMR (200 MHz, D_2O) δ 4.37-4.24 (m, 1H), 4.11-3.87 (m, 3H), 3.27-3.13 (m, 2H), 3.05-2.97 (m, 2H). Mass spectrum (FAB) 236.0 (M + 1, 48%).

Anal. Calcd for $C_7H_{13}N_3O_2S_2 \cdot 3 (C_2HF_3O_2)$: C, 27.04; H, 2.79; N, 7.28; S, 11.11.
Found: C, 27.32; H, 2.91; N, 7.41; S, 11.17.

Example 9

Preparation of 4-((2-amino-4-thiazolyl)methyl)-L-homoalanine

4-((2-amino-4-thiazolyl)methyl)-N α -t-Boc-L-homoalanine t-butyl ester was prepared by the method of Patt et al, (Synth. Commun. 1990, 20 (20), 3097-3102) in 45% overall yield (1.3g) from N-t-Boc-L-2-aminoadipic acid 1-t-butyl ester (Ramsamy et al. Synthesis, 1982, 42-43). The t-Boc and t-butyl ester protecting groups were removed as follows:

To a solution of 1.68g 4-((2-amino-4-thiazolyl)methyl)-N α -t-Boc-L-homoalanine t-butyl ester in 35 mL dioxane was added 1.1 mL triethylsilane and 8 mL 4N hydrochloric acid in

dioxane solution. The mixture was filtered and the solids rinsed with dioxane after stirring for 16 hours at 22°C. The NMR of a crude sample indicated incomplete reaction. Redissolved the crude solid in 20 mL dioxane and treated with 4N hydrochloric acid (5 mL) for 4 hours. The solids were isolated by filtration, dissolved into water, and freeze-dried to yield 1.23g (80%) of 4-((2-amino-4-thiazolyl)methyl)-L-homoalanine as a hygroscopic white solid (Bis-hydrochloride hydrated with 1.4 mol % water and solvated with 0.3 mol % dioxane). Analytical HPLC; Phenomenex C 18, water/methanol/trifluoroacetic acid (95/5/0.1), $k' = 0.34$. ^1H NMR (300 MHz, DMSO- d_6) δ 9.25 (br s, 2H), 8.5 (br s 2H), 6.55 (s, 1H), 3.93 (m, 1H), 2.6 (m, 2H), 1.8 (m, 4H).

Anal. Calcd. for $\text{C}_8\text{H}_{13}\text{N}_3\text{O}_2\text{S} \cdot 2\text{HCl} \cdot 1.4 \text{H}_2\text{O} \cdot 0.3 \text{dioxane}$: C, 32.51; H, 5.99; N, 12.36; S, 9.43; Cl, 20.86. Found: C, 32.28; H, 5.72; N 12.71; S, 9.60; Cl, 20.86.

Example 10

Preparation of N,N'-(1,3-Phenylenebis(methylene))bis(S-methylisothiourca)

To a 0°C stirred solution of 3.30g (25 mmol) m-xylylenediamine (Aldrich Chemical) in 100 mL dichloromethane was added 7.0 mL (52 mmol) benzoylisothiocyanate. The mixture was stirred at 20 °C for 18 h and the solvent was removed under reduced pressure. The crude solids (pale yellow) were suspended in 100 mL 10% sodium hydroxide solution and refluxed for 5 minutes. The mixture was acidified with concentrated hydrochloric acid while still hot, cooled, and then made basic by the addition of ammonium hydroxide. The white solids that precipitated were collected and dried at 60 °C under reduced pressure to a constant weight to yield 4.82 g bis-thiourea intermediate as an off-white solid.

Anal. Calcd. for $\text{C}_{10}\text{H}_{14}\text{N}_4\text{S}_2$: C, 47.22; H, 5.55; N, 22.03; S, 25.10. Found: C, 47.49; H, 5.50; N, 21.81; S, 25.01.

To a solution of 2.54 g (10 mmol) of bis-thiourea intermediate in 25 mL dimethylformamide was added 5.0 mL (80 mmol) iodomethane. The solution was stirred 65 h at 20 °C, the solvent was removed under reduced pressure, and the residue was recrystallized from hot ethanol. The pale yellow crystals were dried under reduced pressure at 60°C to yield 4.28g (81%) N,N'-(1,3-Phenylenebis(methylene))bis(S-methylisothiourca). m.p. = 164-167°C. TLC (one spot on silica gel with 1% ammonium hydroxide in methanol, $R_f = 0.48$). ^1H NMR (300 MHz DMSO/ D_2O) δ 7.5-7.4 (m, 1H), 7.35-7.2 (m, 3H), 4.59 (s, 4H), 2.65 (s, 6H).

Anal.Calcd. for $C_{12}H_{18}N_4S_2 \cdot 1.9 HI$: C, 27.43; H, 3.82; N, 10.66; S, 12.20; I, 45.89.
Found: C, 27.30; H, 3.91; N, 10.57; S, 12.19; I, 45.92.

Example 11

Preparation of N,N'-(1,3-Phenylenebis(methylene))bis(S-ethylisothiourea)

To a solution of 1.0 g (3.93 mmol) of the bis-thiourea prepared in example 10 in 20 mL ethanol was added 6.29 g (78.62 mmol) of iodoethane. The mixture was heated to reflux for 8 h and concentrated to a foam under reduced pressure. The crude product was purified by silica gel chromatography with methanol in dichloromethane (10% to 20%) to yield 1.66g (74%) of N,N'-(1,3-Phenylenebis(methylene))bis(S-ethylisothiourea). TLC (R_f = 0.3-0.48, 20% methanol in dichloromethane). Mass spectrum (FAB) 311.2 (M+1).

1H NMR (200 MHz, DMSO- d_6) δ 7.5-7.4 (m, 1H), 7.31-7.22 (m, 3H), 4.58 (s, 4H), 3.20 (q, J = 7.4 Hz, 4H), 1.28 (t, J = 7.4 Hz, 6H).

Anal. Calcd. for $C_{14}H_{24}N_4S_2 \cdot 2HI$: C, 29.69; H, 4.27; N, 9.89; S, 11.32; I, 44.82. Found : C, 29.44; H, 4.25; N, 9.64; S, 11.50; I, 44.65.

Example 12

Preparation of S-(3-(4-((Amidinothio)methyl)phenyl)propyl)isothiourea

From 3-(4-Carboxyphenyl)propionic acid (Lancaster Synthesis) was prepared 3-(4-((Amidinothio)methyl)phenyl)propyl)isothiourea as a white solid (2.95 g, m.p. = 190-195 ° C) by the method of example 1. Mass spectrum (FAB) 283 (M+1). 1H NMR (200 MHz, D_2O) δ 7.38 (d, J = 8.1 Hz, 2H), 7.26 (d, J = 8.1, Hz, 2H), 4.36 (s, 2H), 3.06 (t, J = 7.2 Hz, 2H), 2.74 (t, J = 7.4 Hz, 2H), 2.1-1.9 (m, 2H).

Anal.Calcd. for $C_{12}H_{18}N_4S_2 \cdot 2HBr$: C, 32.44; H, 4.54; N, 12.61; S, 14.43; Br, 35.97. Found : C, 32.53; H, 4.58; N, 12.56; S, 14.34; Br, 35.85.

Example 13

Preparation of S-(2-(5-((Amidinothio)methyl)-2-thienyl)ethyl)isothiourea

To a solution of 21.48 g (0.19 mol) α , α -dichloromethyl methyl ether (Fluka) in 300 mL dichloromethane at 0°C was added 44.76 g (0.172 mol) tin (IV) chloride (Aldrich). After 15 minutes, a solution of 24.01 g (0.14 mol) ethyl 2-thiopheneacetate (Aldrich) in 50 mL dichloromethane was added dropwise over several minutes. The mixture was poured into

water and ice after 1 h and stirred for 30 minutes. The dichloromethane layer was washed with water, dried over sodium sulfate, and concentrated. The crude product was purified by silica gel chromatography with 10% ethyl acetate in hexanes to yield 22.85 g (82%) of 5-formyl-2-thiopheneacetic acid ethyl ester intermediate.

To a 0°C stirred suspension of 0.77g (20.29 mmol) lithium aluminum hydride (Aldrich) in 200 mL tetrahydrofuran was added a solution of 2.0 g (10.09 mmol) of the intermediate prepared above in 50 mL tetrahydrofuran. The suspension was stirred at 20°C for 16 h, cooled to 0°C, and the excess hydride was quenched by the careful addition of 0.8 mL water, 0.8 mL 1N sodium hydroxide solution, and 2.4 mL water. The suspension was stirred with magnesium sulfate, filtered, concentrated, and purified by silica gel chromatography with 50% ethyl acetate in hexanes to 100% ethyl acetate. There was isolated 1.07 g (67%) of diol intermediate that was converted directly to dibromide as follows.

The diol intermediate (1.07 g, 6.76 mmol) in 50 mL dichloromethane at 0°C was treated with 3.90 g (14.87 mmol) triphenylphosphine and 4.93 g (14.87 mmol) carbon tetrabromide. The solution was stirred for 1 h before 200 mL pentane was added. The supernatant was decanted from an oily residue, concentrated, and purified by silica gel chromatography with hexanes to give 1.10g (57%) of a dibromide intermediate. The dibromide product (1.10g, 3.87 mmol) in 50 mL ethanol was treated with 0.59 g (7.75 mmol) thiourea. The solution was stirred at reflux for 2 h. The concentrated solution was purified by preparative HPLC (Waters C18 BondaPak PrepPak cartridge) with a methanol/water/trifluoroacetic acid gradient (5/95/0.1 to 90/10/0.1). The pooled product fractions were concentrated, diluted with water, and freeze-dried to yield 306 mg of S-(2-(5-((Amidinothio)methyl)-2-thienyl)ethyl)isothiourea as a bis-trifluoroacetic acid salt and 0.1 mol hydrate (mp = 186-190°C). ¹H NMR (200 MHz, D₂O) δ 6.98 (d, J = 3.5 Hz, 1H), 4.57 (s, 2H), 3.38 (t, J = 6.5 Hz, 2H), 3.2 (t, J=6.5 Hz, 2H).

Anal. Calcd. for C₉H₁₄N₄S₃ • 2.0 C₂HF₃O₂ • 0.1 H₂O : C, 30.96; H, 3.24; N, 11.11; S, 19.08. Found: C, 31.02; H, 3.19; N, 11.00; S, 18.97.

Example 14

Preparation of S-(3-(5-(2-(Amidinothio)ethyl)-2-thienyl)propyl)isothiourea

5-Formyl-2-thiopheneacetic acid ethyl ester was prepared as described in example 13. To a solution of 2.0g (10.09 mmol) of this ester in 100 mL tetrahydrofuran was added 3.87 g

(11.10 mmol) carbethoxymethylenetriphenylphosphorane. The solution was refluxed overnight and concentrated. The crude product was combined with a second 2.0g reaction utilizing 10.54g (30.27mmol) carboethoxymethylenetriphenylphosphorane refluxed in 100 mL tetrahydrofuran for 3h. The combined crude products were purified by silica gel chromatography (10% ethyl acetate/hexane) to yield 2.39g (44%) of enediester intermediate. To a solution of 1.0g (3.73 mmol) of this ene-diester in 50 mL ethanol was added 1.0g 10% palladium on carbon. The mixture was shaken at 20°C under 50 psi hydrogen for 14 h. The catalyst was removed by filtration through celite and the solution was concentrated to yield 1.0g of crude diester intermediate. The final three reactions on this intermediate, including lithium aluminum hydride reduction (82%), bromination of the resulting diol (78%), and alkylation of the dibromide with thiourea (88%) were analogous to those described in example 13. The crude product of the alkylation reaction was purified by preparative HPLC (Waters, C18 BondaPak PrepPak cartridge) eluting with a methanol/water gradient from 10% to 90% methanol over 40 minutes (solutions were buffered with 0.1% trifluoroacetic acid). The product fractions were freeze-dried to yield 1.53g of S-((3-(5-(2-(Amidinothio)ethyl)-2-thienyl)propyl)isothiurea as a mixed salt (1.0 HBr, 1.1 TFA) and 0.5 mol of solvation with methanol. M.p. = 146-151°C. Mass spectrum (FAB) 303. (M+1). ¹H NMR (200 MHz, D₂O) δ 6.77 (d, J = 3.3 Hz, 1H), 6.73 (d, J = 3.3 Hz, 1H), 3.37 (t, J = 6.6 Hz, 2H), 3.21-3.03 (m, 2H), 2.91 (t, J = 7.2 Hz, 2H), 2.1-1.95 (m, 2H).

Anal. Calcd, for C₁₁H₁₈N₄S₃ • 1.0 HBr • 1.1 C₂HF₃O₂ • 0.5 H₂O; C, 31.35; H, 4.24; Br, 15.22; N, 10.68; S, 18.05. Found: C, 31.59; H, 4.01; Br, 14.94; N, 10.45; S, 18.05.

Examples 15-17 were prepared by the method of example 2. The crude products were purified by preparative HPLC (Waters, C18 BondaPak PrepPak cartridge). Gradient elutions with methanol/water/trifluoroacetic acid (5/95/0.1 to 90/10/0.1) followed by freeze-drying provided the target amino acids as trifluoroacetic acid addition salts.

Example 15

S-((2-Amino-4-thiazolyl)methyl)-D-cysteine

Prepared from D-cysteine and 2-amino-4-chloromethylthiazole (Sprague, J.M. *et al.* J. Am. Chem. Soc. 1946, 68, 2155-2159). Analytical HPLC - phenomenex C18, water/methanol/heptafluorobutyric acid (80/20/0.17), one peak, k' = 1.9. UV (pH 7.0 buffer) λ_{max} 254 nm (log ε 3.73). ¹H NMR (200 MHz, DMSO-d₆) δ 8.1 (br, 2H), 8.1-7.5 (br, 2H), 6.5 (s, 1H), 4.25 (m, 1H), 3.65 (d, J = 6 Hz, 2H), 3.0 (m, 2H).

Anal.Calcd. for $C_7H_{11}N_3O_2S \cdot 2.3 C_2HF_3O_2 \cdot 0.1 H_2O$; C, 27.92; H, 2.72; N, 8.43; S, 12.85. Found: C, 28.00; H, 2.89; N, 8.74; S, 12.62.

Example 16

S-((2-Amino-4-thiazolyl)methyl)-(D,L)-homocysteine

Prepared from (D,L)-homocysteine and 2-amino-4-chloromethylthiazole (Sprague, J.M. *et al* J.Am.Chem.Soc. 1946, 68, 2155-2159). Analytical HPLC - phenomenex C18, water/methanol/trifluoroacetic acid (95/5/0.1), one peak, $k' = 2.3$. UV (pH 7.0 buffer) λ_{max} 254 nm (log ϵ 3.73). 1H NMR (200 MHz, DMSO- d_6) δ 8.5-8.0 (br, 2H), 7.2-7.0 (br, 2H), 6.35 (s, 1H), 4.0 (t, $J = 4$ Hz, 1H), 3.55 (s, 2H), 2.6 (m, 2H), 2.0 (m, 2H).

Anal.Calcd. for $C_8H_{13}N_3O_2S \cdot 1.1 C_2HF_3O_2 \cdot 1.0 H_2O$; C, 31.35; H, 4.15; N, 10.75; S, 16.41. Found: C, 31.30; H, 4.07; N, 10.73; S, 16.31.

Example 17

S-(2-(2-Amino-4-thiazolyl)ethyl)-L-cysteine

From L-cysteine and 2-amino-4-(2-bromoethyl)thiazole (prepared from 1,5-dibromo-2-butanone by the method of Sprague *et al*, J.Am.Chem.Soc. 1946, 68, 2155-2159). Analytical HPLC - phenomenex C18, water/methanol/trifluoroacetic acid (95/5/01), one peak, $k' = 2.6$. UV (pH 7.0 buffer) λ_{max} 256 nm (log ϵ 3.71). 1H NMR (200 MHz, DMSO- d_6) δ 9.2-8.8 (br, 2H), 8.6-8.2 (br, 2H), 6.6 (s, 1H), 4.2 (m, 1H), 3.0 (m, 2H), 2.8 (m, 4H).

Anal.Calcd. for $C_8H_{13}N_3O_2S_2 \cdot 2.0 C_2HF_3O_2 \cdot 2.4 H_2O$; C, 27.79; H, 3.85; N, 8.10; S, 12.37. Found: C, 27.65; H, 3.69; N, 8.02; S, 12.38.

Example 18

Preparation of S-(2-(4-bromophenyl)ethyl)isothiouraea hydrobromide

To a solution of 4-bromophenethyl alcohol (720mg, 0.50ml, 3.58mmol) and triphenylphosphine (1.13g, 4.30 mmol) in dichloromethane (7.0ml) at 0°C was added carbon tetrabromide (1.42g, 4.30mmol). The reaction mixture was stirred for 30 min while warming to room temperature. The solution was poured into hexane (100ml) and filtered through celite. The solvents were removed *in vacuo*, hexane was added, and the solution

was filtered through celite. After removing the solvent in vacuo, the crude material was kugelrohr distilled (120°C/70µm Hg) to give a clear oil.

The clear oil was dissolved in 95% ethanol (7.0ml), and thiourea (300mg, 3.94 mmol) was added. The reaction mixture was warmed to reflux for 16 hr, cooled to room temperature, and the solvent was removed in vacuo to give a white solid. The solid was suspended in hot acetone and filtered to give 832mg (68% yield) of the title compound.

^1H NMR (300 MHz, d_6 -DMSO); δ 9.02 (s, 4H), 7.52 (d, $J = 8.3$ Hz, 2H), 7.26 (d, $J = 8.3$ Hz, 2H), 3.43 (t, $J = 7.6$ Hz, 2H), 2.91 (t, $J = 7.4$ Hz, 2H).

M.S. (CI) for $\text{C}_9\text{H}_{12}\text{N}_2\text{SBr}_2$, m/z (relative intensity) 259 (M^+-Br , 100), 183 (68), 77 (92).

Elemental Analysis for $\text{C}_9\text{H}_{12}\text{N}_2\text{SBr}_2$, calcd. C, 31.79; H, 3.56; N, 8.24; S, 9.43; Br, 46.99. Found C, 31.84; H, 3.59; N, 8.19; S, 9.34; Br, 46.92.

Example 19

Preparation of S-(2-(4-fluorophenyl)ethyl)isothiurea hydrobromide.

Prepared from 4-fluorophenethyl alcohol according to the method of Example 18. The title compound was purified by recrystallization from absolute ethanol.

^1H NMR (300 MHz, d_6 -DMSO); δ 9.10 (br. s, 2H), 8.96 (br. s, 2H), 7.32 (m, 2H), 7.14 (m, 2H), 3.43 (t, $J = 6.8$ Hz, 2H), 2.91 (t, $J = 6.8$ Hz, 2H).

M.S.(CI) for $\text{C}_9\text{H}_{12}\text{N}_2\text{SFBr}$, m/z (relative intensity) 199 (M^+-Br , 42), 77 (100).

Elemental Analysis for $(\text{C}_9\text{H}_{12}\text{N}_2\text{SF})(\text{CH}_4\text{N}_2\text{S})_{0.63}(\text{HBr})_{1.06}$, calcd. C, 34.84; H, 4.43; N, 13.75; S, 15.74; Br, 25.51. Found C, 35.31; H, 4.41; N, 13.62; S, 15.51; Br, 25.92.

Example 20

Preparation of S-(2-(4-ethoxy-3-methoxyphenyl)ethyl)isothiurea hydrobromide.

Prepared from 4-ethoxy-3-methoxyphenethyl alcohol according to the method of Example 18. The title compound was purified by recrystallization from absolute ethanol.

^1H NMR (300 MHz, d_6 -DMSO); δ 8.95 (br. s, 4H), 6.87 (s, 1H), 6.84 (d, $J = 8.1$ Hz, 1H), 6.73 (d, $J = 8.2$ Hz, 1H), 3.94 (q, $J = 7.0$ Hz, 2H), 3.73 (s, 3H), 3.39 (t, $J = 7.3$ Hz, 2H), 2.83 (t, $J = 7.4$ Hz, 2H), 1.28 (t, $J = 7.0$ Hz, 3H).

M.S. (CI) for $\text{C}_{12}\text{H}_{19}\text{N}_2\text{O}_2\text{SBr}$, m/z (relative intensity) 255 ($\text{M}^+ - \text{Br}$, 56), 179 (100).

Elemental Analysis for $\text{C}_{12}\text{H}_{19}\text{N}_2\text{O}_2\text{SBr}$, calcd. C, 42.99; H, 5.71; N, 8.36; S, 9.56; Br, 23.83. Found C, 43.09; H, 5.73; N, 8.42; S, 9.61; Br, 23.89.

Example 21

Preparation of S-(2-(4-ethoxyphenyl)ethyl)isothioureahydrochloride.

Prepared from 4-ethoxyphenethyl alcohol according to the method of Example 18. The crude product was dissolved in water, and 2 molar equivalents of aqueous sodium picrate was added. The bright yellow precipitate was isolated by filtration and chromatographed on AG1-X2 anion exchange resin (200-400 mesh, chloride form, 10:1 water: methanol eluant) to give the hydrochloride salt.

^1H NMR (300 MHz, d_6 -DMSO); δ 9.19 (br. s, 4H), 7.19 (d, $J = 8.1$ Hz, 2H), 6.82 (d, $J = 8.1$ Hz, 2H), 3.94 (q, $J = 7.0$ Hz, 2H), 3.39 (t, $J = 7.3$ Hz, 2H), 2.83 (t, $J = 7.4$ Hz, 2H), 1.28 (t, $J = 7.0$ Hz, 3H).

M.S. (CI) for $\text{C}_{11}\text{H}_{17}\text{N}_2\text{OSCl}$, m/z (relative intensity) 225 ($\text{M}^+ - \text{Cl}$, 37), 149 (100).

Elemental Analysis for $(\text{C}_{11}\text{H}_{16}\text{N}_2\text{OS})(\text{HCl})_{1.08}(\text{CH}_4\text{N}_2\text{S})_{0.08}(\text{H}_2\text{O})_{0.16}$, calcd. C, 48.81; H, 6.55; N, 11.10; S, 12.70; Cl, 14.04. Found C, 48.91; H, 6.41; N, 11.12; S, 12.55; Cl, 14.20.

Example 22

Preparation of S-(2-(2,6-dimethoxyphenyl)ethyl)isothioureahydrochloride.

Prepared from 2,6-dimethoxyphenethyl alcohol according to the method of Example 21.

^1H NMR (300 MHz, d_6 -DMSO); δ 9.14 (br. s, 4H), 7.18 (t, $J = 8.2$ Hz, 1H), 6.07 (d, $J = 8.2$ Hz, 2H), 3.73 (s, 6H), 3.39 (t, $J = 7.3$ Hz, 2H), 2.83 (t, $J = 7.4$ Hz, 2H).

M.S. (CI) for $\text{C}_{11}\text{H}_{17}\text{N}_2\text{O}_2\text{SCl}$, m/z (relative intensity) 241 ($\text{M}^+ - \text{Cl}$, 25), 165 (100).

Elemental Analysis for $C_{11}H_{17}N_2O_2SCl$, calcd. C, 47.74; H, 6.19; N, 10.12; S, 11.48; Cl, 12.81. Found C, 47.77; H, 6.19; N, 10.07; S, 11.48; Cl, 12.87.

Example 23

Preparation of S-(2-(4-methoxyphenyl)ethyl)isothioureahydrochloride.

Prepared from 4-methoxyphenethyl alcohol according to the method of Example 21.

1H NMR (300 MHz, d_6 -DMSO); δ 9.19 (br, s, 4H), 7.19 (d, J = 8.0 Hz, 2H), 6.82 (d, J = 8.1 Hz, 2H), 3.73 (s, 3H), 3.39 (t, J = 7.3 Hz, 2H), 2.83 (t, J = 7.2 Hz, 2H).

M.S. (CI) for $C_{11}H_{17}N_2OSCl$, m/z (relative intensity) 211 (M^+-Cl , 22), 135 (100).

Elemental Analysis for $(C_{10}H_{15}N_2OSCl)$, calcd. C, 48.68; H, 6.13; N, 11.36; S, 12.89; Cl, 14.45. Found C, 48.70; H, 6.10; N, 11.36; S, 12.89; Cl, 14.45.

Example 24

Preparation of 2-(2-(3-methoxyphenyl)ethyl)isothioureahydrochloride.

Prepared from 3-methoxyphenethyl alcohol according to the method of Example 21.

1H NMR (300 MHz, d_6 -DMSO); δ 9.00 (br, s, 4H), 7.21 (t, J = 7.9 Hz, 1H), 6.80 (m, 3H), 3.72 (s, 3H), 3.43 (t, J = 7.0 Hz, 2H), 2.88 (t, J = 7.4 Hz, 2H).

M.S. (CI) for $C_{10}H_{17}N_2OSCl$, m/z (relative intensity) 211 (M^+-Cl , 100), 135 (10), 77 (70).
Elemental Analysis for $(C_{10}H_{16}N_2OS)(HCl)_{1.05}(CH_4N_2S)_{0.20}(H_2O)_{0.05}$, calcd. C, 45.39; H, 6.13; N, 12.62; S, 14.45; Cl, 13.61. Found C, 45.28; H, 6.18; N, 12.54; S, 14.43; Cl, 13.65.

Example 25

Preparation of S-(2-(2,4,6-trimethylphenyl)ethyl)isothioureahydrobromide.

Prepared from 2,4,6-trimethoxyphenethyl alcohol according to the method of Example 18.
The title compound was purified by recrystallization from absolute ethanol.

^1H NMR (300 MHz, d_6 -DMSO); δ 9.00 (s, 4H), 6.80 (s, 2H), 3.24 (t, $J = 6.8$ Hz, 2H), 2.84 (t, $J = 6.7$ Hz, 2H), 2.49 (s, 3H), 2.28 (s, 6H).

M.S. (CI) for $\text{C}_{12}\text{H}_{19}\text{N}_2\text{SBr}$, m/z (relative intensity) 223 (M^+-Br , 50), 147 (100).

Elemental Analysis for $\text{C}_{12}\text{H}_{19}\text{N}_2\text{SBr}$, calcd. C, 47.53; H, 6.32; N, 9.24; S, 10.57; Br, 26.53. Found C, 47.26; H, 6.30; N, 9.34; S, 10.49; Br, 26.48.

Example 26

Preparation of S-(2-(3-methylphenyl)ethyl)isothiourea hydrobromide.

Prepared from 3-methylphenethyl alcohol according to the method of Example 18. The title compound was purified by recrystallization from absolute ethanol.

^1H NMR (300 MHz, d_6 -DMSO); δ 9.08 (br. s, 2H), 9.03 (br. s, 2H), 7.20 (t, $J = 7.5$ Hz, 1H), 7.08 (m, 3H), 3.43 (t, $J = 7.8$ Hz, 2H), 2.88 (t, $J = 7.6$ Hz, 2H), 2.28 (s, 3H).

M.S. (CI) for $\text{C}_{10}\text{H}_{15}\text{N}_2\text{SBr}$, m/z (relative intensity) 195 (M^+-Br , 100), 119 (56), 77 (42).

Elemental Analysis for $(\text{C}_{10}\text{H}_{15}\text{N}_2\text{SBr})(\text{CH}_4\text{N}_2\text{S})_{0.14}$, calcd. C, 42.60; H, 5.49; N, 11.17; S, 12.79; Br, 27.95. Found C, 42.66; H, 5.50; N, 11.20; S, 12.57; Br, 27.79.

Example 27

Preparation of S-(2-(2-fluorophenyl)ethyl)isothiourea hydrochloride

Prepared from 2-fluorophenethyl alcohol according to the method of Example 21.

^1H NMR (300 MHz, d_6 -DMSO); δ 9.21 (br. s, 4H), 7.39 (m, 1H), 7.28 (m, 1H), 7.17 (m, 2H), 3.43 (t, $J = 7.7$ Hz, 2H), 2.92 (t, $J = 7.8$ Hz, 2H).

M.S. (CI) for $\text{C}_9\text{H}_{12}\text{N}_2\text{SFCl}$, m/z (relative intensity) 199 (M^+-Cl , 100), 123 (39).

Elemental Analysis for $\text{C}_9\text{H}_{12}\text{N}_2\text{SFCl}$, calcd. C, 46.05; H, 5.15; N, 11.93; S, 13.66; Cl, 15.10. Found C, 45.93; H, 5.11; N, 11.83; S, 13.59; Cl, 15.14.

Example 28

Preparation of S-(2-(3-nitrophenyl)ethyl)isothiourea hydrobromide

To a solution of 3-nitrophenethyl alcohol (500mg, 2.99 mmol) and pyridine (35.6mg, 36 μ l, 0.45mmol) in THF (10ml) at 0°C was added phosphorus tribromide (298 mg, 0.10ml, 1.10 mmol). A white precipitate formed immediately. The reaction mixture was warmed to room temperature and stirred for 1 hr. Water and ether were added, and the phases were separated. The organic phase was washed with saturated aqueous sodium bicarbonate and dried over anhydrous magnesium sulfate. The mixture was filtered, and the solvents were removed in vacuo to give a brown oil.

The crude bromide was dissolved in 95% ethanol (10ml), and thiourea (251mg, 3.30 mmol) was added. The reaction mixture was warmed to reflux for 16 hr, cooled to room temperature, and the solvent was removed in vacuo. The crude yellow solid was suspended in acetone, and the mixture was warmed to reflux for 10 min. The hot solution was filtered to give a white solid.

^1H NMR (300 MHz, d_6 -DMSO); δ 9.12 (br. s, 2H), 8.96 (br. s, 2H), 8.22 (s, 1H), 8.14 (d, J = 7.9 Hz, 1H), 7.78 (d, J = 7.6 Hz, 1H), 7.64 (t, J = 8.0 Hz, 1H), 3.50 (t, J = 7.0 Hz, 2H), 3.10 (t, J = 7.2 Hz, 2H).

M.S. (CI) for $\text{C}_9\text{H}_{12}\text{N}_3\text{O}_2\text{SBr}$, m/z (relative intensity) 226 ($\text{M}^+ - \text{Br}$, 26), 136 (36), 76 (81).

Elemental Analysis for $\text{C}_9\text{H}_{12}\text{N}_2\text{O}_2\text{SBr}$, calcd. C, 35.31; H, 3.95; N, 13.72; S, 10.47; Br, 26.10. Found C, 35.22; H, 3.88; N, 13.62; S, 10.36; Br, 26.18.

Example 29

Preparation of S-(2-(1H-pyrrol-1-yl)ethyl)isothiourea

To a solution of freshly distilled N-(2-bromoethyl)pyrrole (1.00g, 5.74 mmol) in 95% ethanol (12ml) at room temperature was added thiourea (415mg, 5.74mmol). The solution was stirred at reflux for 22 hr, cooled to room temperature, and the mixture was concentrated in vacuo to give a thick slightly beige oil. The oil was allowed to crystallize at room temperature over 16 hr, and the crystals were filtered and rinsed with chloroform (10ml) to give 1.078g of the title compound as slightly beige needle-like crystals (75% yield).

^1H NMR (300 MHz, d_6 -DMSO); δ 9.06 (br. s, 4H), 6.80 (t, J = 2.10 Hz, 2H), 5.99 (t, J = 2.10 Hz, 2H), 4.12 (t, J = 6.50 Hz, 2H), 3.52 (t, J = 6.50 Hz, 2H).

M.S. (CI) for $C_7H_{12}N_3SBr$, m/z (relative intensity) 170 (M^+-Br , 100), 153 (13), 103 (14), 94 (14), 93 (10), 77 (24).

Elemental Analysis for $C_7H_{12}N_3SBr$, calcd. C, 33.61; H, 4.84; N, 16.80; S, 12.82; Br, 31.94. Found C, 33.68; H, 4.84; N, 16.74; S, 12.74; Br, 31.99.
m.p. 94.6 - 95.0°C.

Example 30

NO synthase inhibition was determined by the following procedure:

Purification of NOS from human placenta

Amion and chorion were removed from fresh placenta, which was then rinsed with 0.9% NaCl. The tissue was homogenized in a Waring blender in 3 volumes of HEDS buffer (20 mM Hepes pH 7.8, 0.1 mM EDTA, 5mM DTT, 0.2M sucrose) plus 0.1 mM PMSF. The homogenate was filtered through cheesecloth and then centrifuged at 1000g for 20 min. The supernatant was recentrifuged at 27,500g for 30 min. Solid ammonium sulfate was added to the supernatant to give 32% saturation. Precipitated protein was pelleted at 25,000g and then redissolved in a minimal volume of HEDS buffer plus 0.1 mM PMSF, 10 μ g/ml leupeptin and soybean trypsin inhibitor, and 1 μ g/ml pepstatin. The redissolved pellet was centrifuged at 15,000g for 10 min. To the supernatant was added 1/20 volume at 2.5' ADP agarose resin (Sigma), and the slurry was mixed slowly overnight. In the morning, slurry was packed into a column. The resin was sequentially washed with HEDS, 0.5M NaCl in HEDS, HEDS, and then NOS was eluted with 10 mM NADPH in HEDS. The enzyme could be concentrated by ultrafiltration and quick frozen and stored at -70°C without loss in activity for at least 3 months.

Assay for human placental NOS

NOS was assayed for the formation of citrulline following the procedure of Schmidt et al (PNAS 88 365-369, 1991) with these modifications: 20 mM Hepes, pH 7.4, 10 μ g/ml calmodulin, 2.5 mM $CaCl_2$, 2.5 mM DTT, 125 μ M NADPH, 10 μ M tetrahydrobiopterin, 0.5mg/ml BSA, and 1 μ M L-[^{14}C]arginine (New England Nuclear). Linearity of NOS-catalyzed rate was confirmed prior to kinetic studies that used single time point determination of rate.

Purification of NOS from cytokine-induced human colorectal adenocarcinoma DLD-1 cells.

DLD-1 (ATCC No. CCL 221) were grown at 37°C, 5% CO₂ in RPMI 1640 medium supplemented with L- glutamine, penicillin, streptomycin, and 10% heat-inactivated fetal bovine serum. Cells were grown to confluence and then the following cocktail of cytokines were added: 100 units/ml interferon-gamma, 200 units/ml interleukin-6, 10 ng/ml tumor necrosis factor, and 0.5 ng/ml interleukin- 1 β . At 18-24 hr post-induction, cells were harvested by scraping and washed with phosphate-buffered saline. Pelleted cells were stored at -70°C. Purification of the induced NOS was performed at 4°C. Crude extract was prepared by three cycles of freeze/thawing cells in TDGB (20 mM tris pH 7.5, 10% glycerol, 1mM DTT, 2 μ M tetrahydrobiopterin). Extract was applied directly onto a column of 2',5' ADP sepharose (Pharmacia). Resin was sequentially washed with TDGB, 0.5M NaCl in TDGB, TDGB. NOS was eluted with 2 mM NADPH in TDGB. BSA was immediately added to give a final concentration of 1 mg/ml. NOS could be quick frozen and stored at -70°C without loss in activity for at least 2 months.

Assay for inducible human NOS.

The formation of citrulline were assayed as described above except that 10 μ M FAD was included and calmodulin and CaCl₂ excluded from the assay mix.

Purification of NOS from human brain

Human brain NOS was prepared using variations of the procedures of Schmidt et al. (PNAS 88 365-369, 1991), Mayer et al. (Fed. Eur. Biochem. Soc. 288 187-191, 1991), and Bredt and Snyder, (PNAS 87 682-685, 1990). Briefly, fresh whole brains (3 with myelinated tissue dissected away, 1050g) were homogenized in cold buffer A (50 mM HEPES, pH 7.5 (pH at RT) and 0.5 mM EDTA, 10 mM DTT, 3.6 L total volume) with a polytron. The mixture was centrifuged at 13,000g for 1 hour and the supernatant fluid was removed (about 2050ml). To the supernatant fluid, solid ammonium sulfate (365g, about 30% of saturation) was added and stirred slowly for a total of 30 minutes. The precipitate was pelleted at 13,000g for 30 minutes and the pellet was resuspended in ~400ml of buffer A with 4 μ M tetrahydrobiopterin, 1 μ M FAD (Sigma), and 1 μ M FMN (Sigma). The solution was centrifuged at 41,000g for 60 minutes. The supernatant was removed, frozen by pouring

into liquid nitrogen, and stored overnight at -70°C . The mixture was thawed and passed through a 2',5' ADP-agarose column (0.4g swelled in buffer A) at 4ml/min. The column was washed with 100ml buffer A, 200ml buffer A with 500 mM NaCl, 100ml Buffer A, then 30ml buffer A with 5 mM NADPH. To the enzyme solution was added tetrahydrobiopterin to 10 μM , FAD and FMN to 1 μM , and Tween to 0.1%. This solution was concentrated by Centriprep-30 to a volume of approximately 500 μl . Enzyme activity was determined as described by Schmidt et al. 1991, except that 10 μM tetrahydrobiopterin was included in the assay.

The inhibition results are shown in Table 1.

Values are inhibition constants (K_i) obtained from measuring percent inhibition at three or more concentrations of inhibitor and assuming competitive inhibition with respect to arginine.

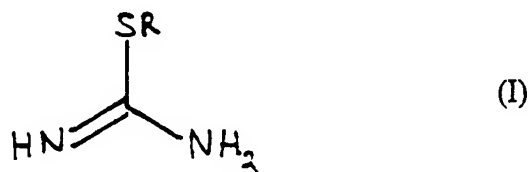
Table 1
Inhibition of Nitric Oxide Synthase

	COMPOUND	HUMAN INDUCIBLE	HUMAN PLACENTAL	HUMAN BRAIN
1	S-(2-aminoethyl)isothiourea	0.59 ± 0.2 µM	2.1 µM	1.8 ± 0.1 µM
2	S-(2-(dimethylamino)propyl)isothiourea	7 ± 1 µM	53 µM	57 ± 5 µM
3	S-(2-methyl-2-propenyl)isothiourea	0.28 µM	0.63 ± 0.18 µM	0.42 ± 0.09 µM
4	S,S'-ethylenebis(isothiourea)	1.4 ± 0.4 µM	1.9 ± 0.2 µM	1.8 ± 0.1 µM
5	S,S'-pentamethylenebis(isothiourea)	5.8 ± 0.2 µM	30 ± 6 µM	13 ± 0.2 µM
6	S-(2-(dimethylamino)ethyl)isothiourea	0.39 ± 0.02 µM	6.1 µM	10 ± 1 µM
7	2-amino-2-thiazoline	0.26 ± 0.03 µM	0.35 ± 0.05 µM	0.41 ± 0.02 µM
8	S,S'-hexamethylenebis(isothiourea)	4.8 ± 0.4 µM	24 µM	
9	S,S'-heptamethylenebis(isothiourea)	0.8 ± 0.3 µM	2.9 ± 0.1 µM	1.7 ± 0.1 µM
10	S-benzylisothiourea	5.6 ± 0.4 µM	23 ± 2 µM	14 ± 2 µM
11	S-(2-morpholinoethyl)isothiourea	19 ± 1 µM	53 µM	
12	S-(6-methyl-2-(methylthio)-4-pyrimidinyl)isothiourea	3.2 ± 0.4 µM	5.4 µM	5.6 ± 0.5 µM
13	S,S'-(1,4-phenylenebis(methylene))diisothiourea	0.12 ± 0.1 µM	2.7 ± 0.1 µM	1.2 ± 0.06 µM
14	S-tertbutylisothiourea	0.24 ± 0.02 µM	1.2 ± 0.04 µM	0.62 ± 0.03 µM
15	S-(4-ethylbenzyl)isothiourea	7.0 ± 0.3 µM	57 ± 8 µM	21 ± 4 µM
16	S-((methylthio)methyl)isothiourea	0.42 ± 0.05 µM	1.5 ± 0.5 µM	0.67 ± 0.05 µM
17	S-(3-bromopropyl)isothiourea	0.34 ± 0.1 µM	1.4 ± 0.1 µM	0.82 ± 0.02 µM

18	S-(2-bromoethyl)isothiouraea	0.49 ± 0.07 µM	1.1 ± 0.06 µM	0.59 ± 0.03 µM
19	S-(3-methyl-2-butenyl)isothiouraea	0.52 ± 0.06 µM	2.3 ± 0.1 µM	2.0 ± 0.1 µM
20	S-allylisothiouraea	0.11 ± 0.01 µM	0.27 ± 0.01 µM	0.22 ± 0.01 µM
21	S-(3-aminopropyl)isothiouraea	0.46 ± 0.01 µM	1.2 ± 0.06 µM	0.61 ± 0.02 µM
22	S,S'-(1,3-phenylenebis(methylene))diisothiouraea	1.5 ± 0.07 µM	23 ± 7 µM	0.71 ± 0.05 µM
23	S,S'-(2-methylene-1,3-propanediyl)diisothiouraea	2.3 ± 0.3 µM	16 ± 2 µM	8.8 ± 0.3 µM
24	S,S'-(2-butyne-1,4-diyl)diisothiouraea	0.47 ± 0.03 µM	6.6 ± 0.8 µM	0.31 ± 0.03 µM
25	S,S'-(1,3-phenylenebis(1,2-ethanediyl))diisothiouraea	0.047 ± 0.003 µM	9.0 ± 0.8 µM	0.25 ± 0.01
26	S,S'-(1,4-phenylenebis(1,2-ethanediyl))diisothiouraea	0.0074 ± 0.0005 µM	0.36 ± 0.02 µM	0.016 ± 0.001 µM
27	2-amino-5-methylthiazole	0.88 ± 0.05 µM	3.0 ± 0.1 µM	1.1 ± 0.1 µM
28	S-((2-amino-4-thiazolyl)methyl)-L-cysteine	0.96 ± 0.03 µM	7.2 ± 0.3 µM	6.8 ± 0.5 µM
29	3-((2-amino-4-thiazolyl)methyl)-L-alanine	4.7 µM	2.2 µM	4.4 ± 0.3 µM
30	2-amino-4-methylthiazole	8.6 ± 0.1 µM	7.7 ± 0.8 µM	10 ± 2 µM
31	2-amino-4, 5-dimethylthiazole	0.7 ± 0.09 µM	0.45 ± 0.05 µM	0.38 ± 0.01 µM
32	S-(2-(1H-pyrral-1-yl)ethyl) isothiouraea	0.83 ± 0.03 µM	5.6 ± 0.2 µM	3 ± 0.3 µM
33	S-(3-hydroxypropyl)isothiouraea	0.26 ± 0.01 µM	0.83 ± 0.01 µM	0.68 ± 0.3 µM
34	S-(2-(phenyl)ethyl)isothiouraea	0.52 ± 0.06 µM	1.9 ± 0.1 µM	0.8 ± 0.3 µM
35	S-(2-(3-methoxyphenyl)ethyl)isothiouraea	0.22 ± 0.03 µM	4 ± 1 µM	0.82 ± 0.03 µM
36	S-ethylisothiouraea	0.017 ± 0.002 µM	0.039 ± 0.003 µM	0.029 ± 0.009 µM
37	S-isopropylisothiouraea	0.0098 ± 0.0007 µM	0.022 ± 0.0005 µM	0.037 ± 0.008 µM
38	S-propylisothiouraea	0.24 ± 0.02 µM	0.67 ± 0.02 µM	0.63 ± 0.07 µM

CLAIMS

- 1) Use of an isothiourea derivative having an inhibitory effect against the NO synthase enzyme for the manufacture of a medicament for the treatment of a condition where there is an advantage in inhibiting the NO synthase enzyme.
- 2) Use according to Claim 1 wherein the isothiourea derivative is a compound of formula (I)



or a salt thereof, wherein

- R is
- (1) a C₁₋₁₄ hydrocarbonyl group; or
 - (2) a 5- or 6-membered heterocyclic ring; or
 - (3) a 9-membered bicyclic heterocyclic ring system

each group R being optionally substituted by one or two groups independently selected from:

- (a) halo;
- (b) -XR¹ wherein

X is oxygen, C(O)_m wherein m is 1 or 2, S(O)_n wherein n is 0, 1, or 2, or NR² wherein R² is hydrogen, C₁₋₆ alkyl or C₃₋₆ cycloalkyl or R² is linked to R¹ to form a C₂₋₆ alkylene group;

R¹ is hydrogen; or C₁₋₆ alkyl, C₂₋₆ alkenyl, C₃₋₆ cycloalkyl, C₇₋₉ aralkyl, C₆₋₁₀ aryl, or a 5- or 6-membered heterocyclic group, each group optionally substituted by one or two groups independently selected from C₁₋₃ alkyl, hydroxy, C₁₋₃ alkoxy, amino, C₁₋₃ alkylamino, halo, nitro, or a group C(O)_{m'} R^{2b} wherein m' is 1 or 2 and R^{2b} is hydrogen or C₁₋₄ alkyl; or R¹ is a group NR³R⁴ wherein

R^3 and R^4 are the same or different and each is hydrogen or C_{1-4} alkyl or R^3 and R^4 are linked to form a C_{2-6} alkylene group;

(c) a group $(Y)_w-Q-S-\text{C}(\text{NH})_2$ wherein

Y is oxygen, $S(O)_n$ wherein n is as hereinbefore defined, or NR^5 wherein R^5 is hydrogen or C_{1-4} alkyl;

w is 0 or 1;

Q is C_{2-4} hydrocarbyl

or the imino nitrogen is linked to the group R or to the group Q to form a 5- or 6-membered heterocyclic ring; or

(d) a group A wherein A is a heterocyclic ring system optionally substituted by a group $(Y)_w-Q-S-\text{C}(\text{NH})_2$ as hereinbefore defined; or

(e) C_{1-6} alkyl, C_{2-6} alkenyl or alkynyl or a C_{3-6} cycloalkyl group;

or one of the carbon atoms in R is linked to the imino nitrogen atom in the compound of formula (I) to form a 5- or 6- membered heterocyclic ring;

with the proviso that R is not methyl.

3) Use according to Claim 2 wherein R is

(1) C_{1-4} alkyl;

(2) C_{2-4} alkenyl;

(3) a group $-(CH_2)_p-\text{C}_6\text{H}_4-(CH_2)_q-CH_3$ wherein p is 1 or 2 and q is 0 or 1; or

(4) a 5- or 6-membered heterocyclic ring containing one or two nitrogen atoms.

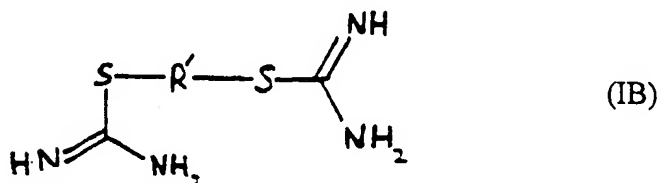
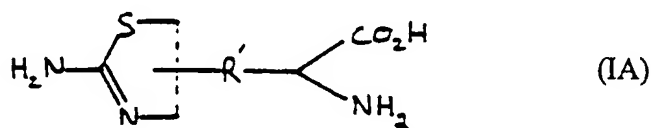
each optionally substituted by one or two groups, which may be the same or different, selected from

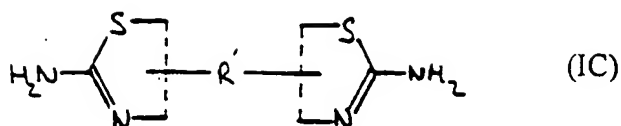
(a) halo, preferably bromo;

- (b) a group $OR^{2b'}$ wherein $R^{2b'}$ is hydrogen or methyl;
- (c) a group $C(O)_m R^{2b'}$ wherein m and $R^{2b'}$ are as hereinbefore defined;
- (d) a group SR^9 wherein R^9 is methyl or ethyl;
- (e) a group $NR^{7b} R^{8b}$ wherein R^{7b} and R^{8b} are independently selected from hydrogen or C_{1-4} alkyl, preferably hydrogen, methyl or ethyl;
- (f) a phenyl ring optionally substituted by a group $OR^{2b'}$ or a group $Q-S-\begin{array}{c} NH \\ \diagup \\ NH_2 \end{array}$ as hereinbefore defined;
- (g) a 5- or 6-membered heterocyclic ring containing one or two heteroatoms independently selected from nitrogen or oxygen; or
- (h) C_{1-4} alkyl, preferably methyl

or one of the carbon atoms in R is linked to the imino nitrogen in the compound of formula (I) to form a thiazole or thiazoline ring.

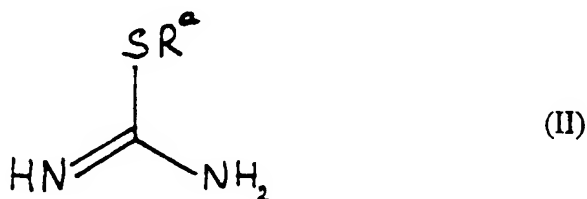
- 4) Use according to Claims 2 or 3 with the proviso that R is not ethyl, propyl or isopropyl.
- 5) Use according Claims 1 to 2 wherein the isothiurea derivative is a compound of formula (IA), (IB) or (IC).



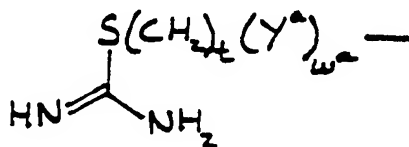


wherein R' is a C₁₋₈ alkylene group, C₂₋₈ alkenylene or alkynylene group each optionally containing a phenyl ring, a 5- or 6-membered heterocyclic ring or a group X as hereinbefore defined, and the dotted line represents a double or a single bond.

- 6) Use according to Claim 1 wherein the isothioureia derivative is a compound of formula (II)



or a salt thereof, wherein R^a is a C₁₋₈ hydrocarbyl or 5- or 6-membered heterocyclic ring or a 9-membered bicyclic heterocyclic ring system each optionally substituted by halo or by one or two groups -X^aR^{1a} wherein R^{1a} is hydrogen, C₁₋₆ alkyl, C₃₋₆ cycloalkyl, C₇₋₉ aralkyl, C₆₋₁₀ aryl, or a 5- or 6-membered heterocyclic group each optionally substituted by C₁₋₃ alkyl, C₁₋₃ alkoxy, amino, halo or nitro or R^{1a} is a group NR^{3a}R^{4a} wherein R^{3a} and R^{4a} are the same or different and each is hydrogen or C₁₋₃ alkyl or R^{3a} and R^{4a} are linked to form a C₂₋₆ alkylene group and X^a is oxygen, C(O)_{m^a} wherein m^a is 1 or 2, S(O)_{n^a} wherein n^a is 0, 1 or 2 or NR^{2a} wherein R^{2a} is hydrogen, C₁₋₆ alkyl or C₃₋₆ cycloalkyl or R^{2a} is linked to R^{1a} to form a C₂₋₆ alkylene group, or by a group



wherein t is 0 to 4 and w^a is 0 or 1, Y^a is oxygen, sulphur and NR^{7a} wherein R^{7a} is hydrogen or C₁₋₄ alkyl:

or R^a links the sulphur atom to one of the nitrogen atoms in the compound of the formula (I) to form a 5- or 6-membered heterocyclic ring, with the proviso that R^a is not methyl.

- 7) Use according to any of Claim 1 to 6 wherein the isothioureia derivative is selected from

S-(2-aminoethyl)isothioureia
S-(2-(dimethylamino)propyl)isothioureia
S-(2-methyl-2-propenyl)isothioureia
S,S'-ethylenebis(isothioureia)
S,S'-pentamethylenebis(isothioureia)
S-(2-(dimethylamino)ethyl)isothioureia
2-amino-2-thiazoline
S,S'- hexamethylenebis(isothioureia)
S,S'- heptamethylenebis(isothioureia)
S-benzylisothioureia
S-(2-morpholinoethyl)isothioureia
S-(6-methyl-2-(methylthio)-4-pyrimidinyl)isothioureia
S,S'-(1,4-phenylenebis(methylene))diisothioureia
S-tertbutylisothioureia
S-(4-ethylbenzyl)isothioureia
S-((methylthio)methyl)isothioureia
S-(3-bromopropyl)isothioureia
S-(2-bromoethyl)isothioureia
S-(3-methyl-2-butenyl)isothioureia
S-allylisothioureia
S-(3-aminopropyl)isothioureia
S,S'-(1,3-phenylenebis(methylene))diisothioureia
S,S'-(2-methylene-1,3-propanediyl) diisothioureia
S,S'-(2-butyne-1,4-diyl)diisothioureia
S,S'-(1,3-phenylenebis(1,2-ethanediyl))diisothioureia
S,S'-(1,4-phenylenebis(1,2-ethanediyl))diisothioureia
2-amino-5-methylthiazole
S-((2-amino-4-thiazolyl)methyl-L-cysteine
3((2-amino-4-thiazolyl)methyl-L-alanine
2-amino-4-methylthiazole
2-amino-4,5-dimethylthiazole
S-(2-(1H-pyrrol-1-yl)ethyl)isothioureia
S-(3-hydroxypropyl)isothioureia

S-(2-(phenyl)ethyl)isothiouraea
S-(2-(3-methoxyphenyl)ethyl)isothiouraea
4-((2-amino-4-thiazolyl)methyl)-L-homoalanine
N,N-1,3-phenylenebis(methylene))bis(S-methylisothiouraea)
N,N-(1,3-phenylenebis(methylene))bis(S-ethylisothiouraea)
S-(2-(5-((amidinothio)methyl)-2-thienyl)ethyl)isothiouraea
S-(3-(4-((amidinothio)methyl)phenyl)propyl)isothiouraea
S-(3-(5-(2-amidinothio)ethyl)-2-thienyl)propyl)isothiouraea
S-(2-(4-fluorophenyl)ethyl)isothiouraea
S-(2-(4-bromophenyl)ethyl)isothiouraea
S-(2-(3-methoxyphenyl)ethyl)isothiouraea
S-(2-(3-methylphenyl)ethyl)isothiouraea
S-(2-(4-ethoxyphenyl)ethyl)isothiouraea
S-(2-(4-methoxyphenyl)ethyl)isothiouraea
S-(2-(2-bromophenyl)ethyl)isothiouraea
S-(2-(2-fluorophenyl)ethyl)isothiouraea
S-(2-(3-nitrophenyl)ethyl)isothiouraea
S-(3-(1H-pyrrol-1-yl)propyl)isothiouraea
S-(2-(2-chlorophenyl)ethyl)isothiouraea
S-(2-(2,5-dimethylphenyl)ethyl)isothiouraea
S-(2-(4-ethoxy-3-methoxyphenyl)ethyl)isothiouraea

or a salt thereof.

- 8) Use according to any of Claims 1 to 3 or 6 wherein the isothiouraea derivative is selected from

S-ethylisothiouraea
S-propylisothiouraea
S-isopropylisothiouraea.

- 9) Use of an isothiouraea according to Claim 1 for the treatment of systemic hypotension.
- 10) Use of an isothiouraea according to Claim 1 or 2 for the treatment of Septic Shock.

- 11) Use of an isothiurea according to Claim 9 wherein the systemic hypotension is caused by cytokine or cytokine-inducing therapy.
- 12) Use of an isothiurea according to Claim 1 for the treatment of short term immunosuppression.
- 13) Use of an isothiurea according to Claim 1 for the treatment of an autoimmune disease.
- 14) Use of an isothiurea according to Claim 1 for the treatment of an inflammatory condition.
- 15) An isothiurea derivative of formula(I) other than S-ethylisothiurea, S-propylisothiurea, S-isopropylisothiurea, benzylisothiurea, S,S-(1,4-phenylenebis(methylene))diisothiurea and S-(2-(dimethylamino)ethyl)isothiurea for use in medicine.
- 16) A novel isothiurea derivative of formula (IA), (IB) or (IC) as hereinbefore defined.
- 17) An isothiurea derivative according to Claim 16 which is selected from

S,S'-(1,4-phenylenebis(1,2-ethanediyl))diisothiurea

S-(2-(1H-pyrrol-1-yl)ethyl)isothiurea

S-((2-amino-4-thiazolyl)methyl)-L-cysteine

γ -(2'-amino-4-thiazolyl)-L-homoalanine

S,S'-(1,2-phenylenebis(1,2-ethanediyl))diisothiurea

β -(2'-amino-4'-thiazolyl)-L-alanine

S-(2'-amino-5'-(R,S)-thiazolinylmethyl)-L-cysteine

4-((2-amino-4-thiazolyl)methyl)-L-homoalanine

N,N-(1,3-phenylenebis(methylene))bis(S-methylisothiurea)

N,N-(1,3-phenylenebis(methylene))bis(S-ethylisothiurea)

S-(3-(4-((amidinothio)methyl)phenyl)propyl)isothiurea

S-(2-(5-((amidinothio)methyl)-2-thienyl)ethyl)isothiurea

S-(3-(5-((2-amidinothio)ethyl)-2-thienyl)propyl)isothiurea

S-((2-amino-4-thiazolyl)methyl)-D-cysteine

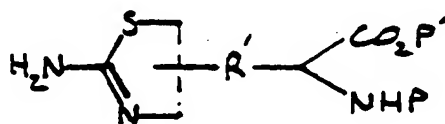
S-((2-amino-4-thiazolyl)methyl)-(D,L)-homocysteine

S-(2-(2-amino-4-thiazolyl)ethyl)-L-cysteine
 S-(2-(4-fluorophenyl)ethyl)isothiourea
 S-(2-(4-bromophenyl)ethyl)isothiourea
 S-(2-(3-methoxyphenyl)ethyl)isothiourea
 S-(2-(3-methylphenyl)ethyl)isothiourea
 S-(2-(4-ethoxyphenyl)ethyl)isothiourea
 S-(2-(4-methoxyphenyl)ethyl)isothiourea
 S-(2-(2-bromophenyl)ethyl)isothiourea
 S-(2-(2-fluorophenyl)ethyl)isothiourea
 S-(2-(3-nitrophenyl)ethyl)isothiourea
 S-(3-(1H-pyrrol-1-yl)propyl)isothiourea
 S-(2-(4-ethoxy-3-methoxyphenyl)ethyl)isothiourea
 S-(2-(2,4,6-trimethylphenyl)ethyl)isothiourea
 S-(2-(2,6-dimethoxyphenyl)ethyl)isothiourea

and salts thereof.

18) A process for the preparation of an isothiourea derivative according to Claim 16 which comprises

- a) the reaction of thiourea with a compound $RL(L')_r$ wherein R is as hereinbefore defined, L and L' are both leaving groups and r is 0 or 1 followed by deprotection if necessary; or
- b) by deprotection of a compound of formula (IB1)



wherein R' and the dotted line are as hereinbefore defined, and P and P' are the same or different and are both protecting groups.

19) A pharmaceutical formulation which comprises an isothiourea derivative as hereinbefore defined other than S-ethylisothiourea, S-propylisothiourea, S, isopropylisothiourea, S-benzylisothiourea, S,S-(1,4-phenylenebismethylene) diisothiourea and S-(2-(dimethylamino)ethyl) isothiourea or a pharmaceutically

acceptable salt or solvate thereof, together with one or more pharmaceutically acceptable carriers therefor and optionally one or more other therapeutic ingredients.

- 20) A method of treatment of a condition where there is an advantage in inhibiting the NO synthase enzyme which comprises administering a therapeutically effective amount of an isothioureia derivative having an inhibitory effect against the NO synthase enzyme.



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/GB93/02437 (22) International Filing Date: 26 November 1993 (26.11.93) (30) Priority Data: 9224948.1 27 November 1992 (27.11.92) GB 9315159.5 22 July 1993 (22.07.93) GB 9319663.2 23 September 1993 (23.09.93) GB (71) Applicant (for all designated States except US): THE WELL- COME FOUNDATION LIMITED [GB/GB]; Unicorn House, 160 Euston Road, London NW1 2BP (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): GARVEY, Edward, Patrick [US/US]; 6418 Heartwood Road, Chapel Hill, NC 27516 (US). TANOURY, Gerald, Joseph [US/US]; 4627-F Hope Valley Road, Durham, NC 27707 (US). OPLINGER, Jeffrey, Alan [US/US]; 337 Bond Lake Drive, Cary, NC 27513 (US). FURFINE, Eric, Steven [US/US]; 4133 Livingstone Place, Durham, NC 27707 (US). (74) Agent: ROLLINS, Anthony, John; The Wellcome Foundation Limited, Langley Court, Beckenham, Kent BR3 3BS (GB).		(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the</i> <i>claims and to be republished in the event of the receipt of</i> <i>amendments.</i> (88) Date of publication of the international search report: 08 December 1994 (08.12.94)	
(54) Title: ENZYME INHIBITORS			
(57) Abstract			
Isothiourea derivatives and their use in medicine, particularly in the treatment of conditions where there is an advantage in inhibiting nitric oxide synthase, pharmaceutical formulations comprising the same and processes for the preparation thereof are disclosed.			

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INTERNATIONAL SEARCH REPORT

International Application No

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A. CLASSIFICATION OF SUBJECT MATTER

IPC 5 A61K31/155 A61K31/00 A61K31/425 A61K31/54 C07D277/18
C07D277/40 C07C335/32

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 A61K C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Derwent Publications Ltd., London, GB; AN 92-259284 & US,A,7 759 999 (US DEPT HEALTH & HUMAN SERVICE) 15 April 1992 see abstract ---	1-20
X	'The Merck Index, 11th Edition', MERCK & CO., INC., RAHWAY, N.J., U.S.A. see page 79, monographs 494 and 498, 2-Aminothiazole and Amiphenazole see page 74, monograph 462, 2-Amino-4-methylthiazole ---	1-20
X	US,A,3 954 982 (BLACK ET AL.) 4 May 1976 see column 1, line 13 - line 17 see column 3, formula IV ---	1-20
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☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

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 "O" document referring to an oral disclosure, use, exhibition or other means
 "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

8 August 1994

Date of mailing of the international search report

02. 11. 94

Name and mailing address of the ISA

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 420 396 (SMITH KLINE & FRENCH LABORATORIES LIMITED) 3 April 1991 see page 4, line 37 - line 42 ---	1-20
X	US,A,4 126 670 (DURANT ET AL.) 21 November 1978 see the whole document ---	15
X	EP,A,0 245 669 (FLOERSHEIM, GEORG. L. PROF. DR.) 19 November 1987 see claim 12 ---	15
X	DE,A,23 17 106 (UNIROYAL, INC.) 11 October 1973 see claims 1,2 ---	15
X	US,A,4 262 125 (KLAUBERT) 14 April 1981 see the whole document ---	15
X	US,A,4 208 430 (BONDINELL ET AL.) 17 June 1980 see the whole document ---	15
X	US,A,3 790 600 (Z.S.ARIYAN ET AL.) 5 February 1974 see column 1 ---	15
X	J.MOL.GRAPH., vol.10, no.2, June 1992 pages 79 - 87 E.E.J.HAAKSMA ET AL. 'A theoretical study concerning the mode of interaction of the histamine H2-agonist dimaprit' see page 84, right column - page 85, left column ---	15
X	AGENTS ACTIONS, vol.18, no.1-2, April 1986 pages 137 - 140 G.J.STERK ET AL. 'The influence of guanidino and isothioureas groups in histaminergic compounds on H2-activity' see page 138; table IA see page 140; table IIB ---	15
X	INDIAN J. EXP. BIOL., vol.19, no.12, December 1981 pages 1150 - 1153 K.NAGARAJAN ET AL. 'Structure Activity Relations among Cyclic & Acyclic S-(3-Indolyl)isothioureas - Development of a Potent Vasoconstrictor, Tinazole, 3-(2-Imidazolin-2-yl-thio)indole' see the whole document ---	15

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YAKUGAKU ZASSHI, vol.100, no.12, 1980 pages 1225 - 1234 T.HIRAYAMA ET AL. 'Anti-tumor Activities of Some Heterocyclic and Nitrogen-containing Compounds' see abstract ---	15
X	J.MED.CHEM., vol.24, no.8, August 1981 pages 913 - 920 R.GANELLIN 'Medicinal Chemistry and Dynamic Structure-Activity Analysis in the Discovery of Drugs Acting at Histamine H2 Receptors' see page 914; table I see page 915, left column, compound 4 ---	15
X	ARZNEIMITTELFORSCHUNG, vol.35, no.1A, 1985 pages 390 - 394 S.FRENCH ET AL. 'Modulation of in vitro Cellular Immune Response by Histamine Agonists or Antagonists in Murine Species' see abstract ---	1-20
X	BIOCHEM. PHARMACOL., vol.43, no.10, 28 May 1992 pages 2083 - 2090 G.A.FECHNER ET AL. 'ANTIPROLIFERATIVE AND DEPIGMENTING EFFECTS OF THE HISTAMINE (H2) AGONIST DIMAPRIT AND ITS DERIVATIVES ON HUMAN MELANOMA CELLS' see page 2084; figure 1 ---	15
X	AGENTS ACTIONS, vol.7, no.1, March 1977 pages 39 - 43 G.J.DURANT ET AL. 'Dimaprit, [S-[3-(N,N-di methylamino)propyl]isothiurea], A Highly Specific Histamine H2-Receptor Agonist. Part 2. Structure-Activity Considerations' see page 42; table I ---	15
X	BR.J.PHARMACOL., vol.61, no.1, September 1977 pages 101 - 107 S.B.FLYNN ET AL. 'THE CARDIOVASCULAR RESPONSE TO DIMAPRIT, A SELECTIVE HISTAMINE H2-RECEPTOR AGONIST' see abstract ---	1-20
3 3 X	GB,A,1 178 242 (THE WELLCOME FOUNDATION LIMITED) 21 January 1970 see the whole document ---	1-20

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	J.VASC.RES., vol.30, no.3, May 1993 pages 132 - 138 M.KELM ET AL. 'Mechanisms of Histamine-Induced Coronary Vasodilation: H1-Receptor-Mediated Release of Endothelium-Derived Nitric Oxide' see abstract ---	1-20
X	J.PHARMACOL.EXP.THER., vol.260, no.2, February 1992 pages 762 - 767 J.L.ORTIZ ET AL. 'Histamine Receptors on Human Isolated Pulmonary Arterial Muscle Preparations: Effects of Endothelial Cell Removal and Nitric Oxide Inhibitors' see abstract ---	1-20
X,P	NAUNYN-SCHMIEDEBERG'S ARCH. PHARMACOL., vol.347, no.1, January 1993 pages 55 - 60 B.MALINOWSKA ET AL. 'Identification of endothelial H1, vascular H2 and cardiac presynaptic H3 receptors in the pithed rat' see page 57, right column - page 58, left column ---	1-20
A,P	EP,A,0 547 558 (WASHINGTON UNIVERSITY) 23 June 1993 see column 1 - column 4 ---	1-20
X	WO,A,91 04037 (J.K.HELLSTRAND ET AL.) 4 April 1991 see page 3 ---	1-20
X	DIALOG INFORMATION SERVICES, FILE 155: MEDLINE 'accession number 05837693' & INT. ARCH. ALLERGY APPL. IMMUNOL., vol.79, no.3, 1986 pages 249 - 252 L.BEAULIEU ET AL. ---	1-20
X	DIALOG INFORMATION SERVICES, FILE 155: MEDLINE 'accession number 05268819' & PATOL. FIZIOL. EKSP. TER., no.1, January 1984 pages 41 - 45 S.A.SELEZNEV ET AL. see abstract see abstract --- -/--	1-20

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 93/02437

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DIALOG INFORMATION SERVICES, FILE 155: MEDLINE 'accession number 04916379' & AGENTS ACTIONS, vol.12, December 1982 pages 691 - 698 M.R.VICKERS ET AL. see abstract ---	1-20
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INTERNATIONAL SEARCH REPORT

International application No.

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Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claim 20 is directed to a method of treatment of the human/animal body the search has been based on the alleged effects of the compounds.
2. ☒ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
For further information please see annex.
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

For further information please see annex.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

8 and partially 1-7, 9-20.

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

Continuation of BOX I.2:

The expression "an isothiourea derivative" is not a clear and limited description of a chemical compound, because it leaves the complete nature of the remaining part of the molecule open. By the use of this open-end expression a complete search would involve a major part of the chemistry-related IPC documentation. Such a search is economically not feasible.

The further definition of that expression as "having an inhibitory effect against the NO synthase enzyme", expressing a limitation by means of a pharmacological/biological activity, would necessitate testing of all known isothiourea derivatives for this activity. The IPEA is not in the position to perform such tests.

The definition of the therapeutic application as "treatment of a condition where there is an advantage in inhibiting the NO synthase enzyme" is not a proper definition of the intended therapeutic use, because it is not fully known which conditions fulfill this requirement, and which conditions do not fulfill this requirement. This has as an effect that in order to judge whether a known isothiourea compound having a pharmaceutical utility would fulfill the requirements as expressed in claim 1, not only the compound itself would have to be tested for possible inhibitory effects against the NO synthase enzyme, but also tests should be performed in order to establish whether the treatment of each disease in question would benefit from inhibiting the NO synthase enzyme.

It is clear that in this situation a complete search is virtually impossible.

The expressions "a heterocyclic ring system", "a 5- or 6-membered heterocyclic ring", "a 5- or 6-membered heterocyclic group" and "a 9-membered bicyclic heterocyclic ring system" leave the nature of the ring, group and ring system open, and hence do not represent full and limited definitions.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

Continuation of BOX II:

1. Claims: 8, and partially 1-7, 9-20: Use of isothioureia derivatives having a pendant amidinothio substituent, and having R representing a hydrocarbyl group for the manufacture of a medicament for the treatment of systemic hypotension, septic shock, short term immunosuppression, autoimmune disease, and inflammatory condition;
2. Claims: partially 1-7, 9-20: Use of isothioureia derivatives having a pendant amidinothio substituent, and having R representing a 5- or 6-membered heterocyclic ring for the manufacture of a medicament for the treatment of systemic hypotension, septic shock, short term immunosuppression, autoimmune disease, and inflammatory condition;
3. Claims: partially 1-2, 4-6, 9-20: Use of isothioureia derivatives having a pendant amidinothio substituent, and having R representing a 9-membered bicyclic heterocyclic ring system for the manufacture of a medicament for the treatment of systemic hypotension, septic shock, short term immunosuppression, autoimmune disease, and inflammatory condition;
4. Claims: partially 1-7, 9-20: Use of 2-amino-1,3-thiazole derivatives, not further condensed with other rings for the manufacture of a medicament for the treatment of systemic hypotension, septic shock, short term immunosuppression, autoimmune disease, and inflammatory condition;
5. Claims: partially 1-2, 4-6, 9-20: Use of 2-amino-1,3-thiazine derivatives, not further condensed with other rings for the manufacture of a medicament for the treatment of systemic hypotension, septic shock, short term immunosuppression, autoimmune disease, and inflammatory condition;
6. Claims: partially 1-2, 4-6, 9-20: Use of other heterocyclic compounds for the manufacture of a medicament for the treatment of systemic hypotension, septic shock, short term immunosuppression, autoimmune disease, and inflammatory condition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

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